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PHYTOPATHOLOGY

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-THIRD ANNUAL MEETING OF THE SOCIETY, DALLAS, TEXAS, DECEMBER 29, 1941, TO JANUARY 1, 1942

The Relation of Potassium to Water-soaking of Tobacco. ALLINGTON, W. B., AND JAMES JOHNSON. The predisposing effect of a low potash nutrition of plants on the severity of certain diseases has long been known or suspected. Studies on physiological water-soaking have shown that this condition also is an important predetermining factor for natural infection with the organisms of tobacco blackfire (*Bacterium angulatum*) and wildfire (*B. tabacum*). Continued investigation of physiological water-soaking has shown that tobacco plants of field size, which have developed or are approaching potassium-deficiency symptoms, water-soak readily; while plants of a similar size, with ample potassium, water-soak only with great difficulty under conditions of high relative humidity and low light intensity. Certain other factors such as nitrogen supply may play an important part in water-soaking as well. Potassium-starved plants, therefore, are predisposed to water-soaking and, subsequently, to bacterial infection. A shortage of potassium in leaf tissue is known to induce necrosis of isolated cells and finally large groups of cells. Since living tissue surrounding necrotic areas in leaves has repeatedly been observed to water-soak more readily than other tissues, this behavior may explain water-soaking when influenced by low potassium. (Wisconsin Agricultural Experiment Station and Division of Tobacco Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture, cooperating.)

Two Leaf-spot Diseases on Sorghum and Related Grasses. BAIN, D. C., AND C. W. EDGERTON. A leaf-spot disease caused by an apparently undescribed fungus has been found on sorghum, Johnson grass, Sudan grass, and a variety of sugar cane (C.P. 33–243) in Louisiana; on sorghum and Johnson grass in Mississippi; and on Sudan grass at Arlington Farm, Virginia. The disease shows in the form of large zonate spots. Spores in pinkish masses are produced in great abundance. The fungus has been isolated from leaves and seeds and the disease has been produced by inoculation. The fungus does not fit well into any of the described genera. Tentatively, as suggested by C. L. Shear, it is being placed in the new genus *Gleocercospora*. This fungus has been confused with *Titaeospora andropogonis* in this country (Pl. Dis. Rptr. 25: 142. 1941), and possibly in plant disease reports from other parts of the world. *T. andropogonis* has been observed on Johnson grass in Louisiana and Mississippi.

New Species of Sphaceloma on Myrtaceae. BITANCOURT, A. A. Since the discovery of *Elsinoë pitangae* on Surinam cherry (*Eugenia pitanga*) the imperfect stage (*Sphaceloma*) of the genus has been found on other fruit trees of this family of phanerogams, *i.e.*, *jaboticaba* (*Myrciaria jaboticaba*) and guava (*Psidium guajava*) both collected by the writer in the State of São Paulo, Brazil, and on plants native to southern Chile, murta (*Ugni lanceolata*) and arrayán (*Myrceugenia apiculata*), collected by S. Arentsen. On the five hosts the pathogens cause the anthracnose type of lesion. The other type of disease caused by the Elsinoaceae is referred to under the more or less common term "scab." Scab of *Eucalyptus* spp., however, a hitherto unreported disease of this important member of the Myrtaceae, appears not to be caused by this group of pathogens. A species of *Coniothecium* was isolated from the diseased tissues.

Antagonism between Strains of the Peach-mosaic Virus in Western Colorado. BODINE, E. W. Preliminary studies of the strains of the peach-mosaic virus in western Colorado show definite indications of "antagonism" between strains. An Elberta peach tree affected with the slight strain of the virus when re-inoculated with buds of the severe strain in the fall of 1938 showed only symptoms of the slight strain the following spring. After buds from the re-inoculated tree had been inserted into 20 healthy Elberta test trees in the fall of 1939, 10 of these trees showed symptoms of the slight strain the spring following inoculation. The remaining 10 trees showed very slight or no symptoms. The inserted buds made growth unions in 18 cases. Ten of these inoculated trees, 5 trees expressing symptoms of the slight strain and 5 showing very slight to no symptoms were then re-inoculated with the severe strain of the virus in the spring and the fall of 1940. In the spring of 1941, all 10 cases expressed only the symptom apparent in each tree in 1940. The remaining 8 inoculated trees used as controls showed the same symptoms as those manifested in 1940. For each year's study adequate control inoculations were made in order to insure that the virus of severe mosaic was introduced in each instance. Alternate buds were taken from the same bud stick for both the control and re-inoculation tests.

Environment and Plant Disease in the Far Southwest. BROWN, J. G. The far Southwest is a region of contrasts. Elevations vary from approximately sea-level to two or more miles above; extremes of temperature range from – 30° F. to 120° F. or even higher; annual precipitation fluctuates between 3 and 20 inches; soils, predominantly alkaline, may be neutral or somewhat acid at the higher elevations and, physically, range from porous to impervious and from almost pure pulverized granitic rock to clays and loams satisfactorily supplied with organic matter; air movement, predominantly toward the west in large masses in summer and toward the east in winter, becomes locally very complex because of the rough topography and the alternation of vegetative cover with bare soil; evaporation exceeds precipitation in the ratio of 5: 1 to 29: 1. These environmental factors may exert a marked influence on the kinds, prevalence and destructiveness of plant disease. This paper presents a few of the many striking examples that might be given to illustrate relations between environmental factors and plant disease in this Southwest country of contrasts.

Gross Pathogenic Effects of Pythium graminicolum, Pythium debaryanum, and Helminthosporium sativum on Seedlings of Crested Wheatgrass. BUCHHOLTZ, W. F. When placed in steamed soil above the seed, immediately below the seed, 1 inch, and 2 inches below the seed of crested wheatgrass (*Agropyron cristatum*), *Pythium debaryanum* interrupted germination, except at 2 inches below the seed, apparently had little or no effect on seedlings. *Helminthosporium sativum* interrupted germination when near the seed; occasionally, at 1-inch and 2-inch depths, was variable in its effect on seedlings, but tended to be somewhat like *P. debaryanum*. *Pythium graminicolum* in all cases interrupted germination, pruned roots, and induced stunting and death of seedlings. When placed in steamed soil alongside rows of seedlings up to 1 month of age, *P. graminicolum* killed seedlings up to 3 weeks of age, induced wilting and stunting of seedlings of all ages. *P. debaryanum* and *H. sativum* killed only occasional seedlings 1 week old or less, induced no detectable wilting or stunting. In brome-grass sod, 1 inch below the seed, *P. graminicolum* interrupted germination, pruned roots, and induced wilting, stunting, and death of seedlings. *P. debaryanum* and *H. sativum* induced no detectable symptoms. The symptoms induced by *P. graminicolum* in these experiments were typical of seedling blight of crested wheatgrass in field soil. *P. graminicolum* is considered to be the cause of the disease. Isolates of *Fusarium* spp. in preliminary trials were less pathogenic than any of the 3 fungi used in these comparisons.

Necrotic Spot, a Peach Disease Transmissible by Budding. CATION, DONALD. A Windsor cherry tree showing no symptoms, budded to Elberta and Carmen peach trees in 1939, resulted in foliar necrotic spots in 1940. Inoculations in 1940 from cherry to peach and from peach to peach resulted in similar symptoms in Hale, Golden Jubilee, South Haven, and Halehaven. Faint chlorotic spots, accompanied by mild leaf distortion, slightly retarded foliation, and a trace of spot necrosis were evident on several varieties in the early season of 1941. Affected leaves were most evident in mid-July. At that time light-brown, dead membranous areas appeared in unfolding leaves. The affected areas soon fell out, leaving clean-edged holes, and the leaves remained green and persisted throughout the remainder of the season. Die back, cortical necrosis, and ring spotting were not evident, differentiating the disease from the ring spot of peach reported by Cochran and Hutchins (Phytopathology **31**: 860, 1940). Trees infected in 1940 showed some, but proportionately fewer, affected leaves in 1941.

Variability in Response of Flax to Seed Treatment. CHRISTENSEN, J. J., AND M. B. MOORE. From 1936 to 1941 about 700 lots of flax seed obtained from farmers in Minnesota were tested for response to fungicides. Yield tests were made at University Farm and at 4 branch stations. In most cases, seed treatment increased the stand and vigor of seedlings and often increased yield, although in some cases it resulted in a decrease. The value of seed treatment varied greatly with the season and with seed lots of the same variety. Seed of the same variety produced in 5 regions in Minnesota during the same season responded quite differently to seed treatment. Different seed lots of the same variety, whether treated or not, possessed different yielding abilities. The results indicate that the value of seed treatment depends on variety, source, and condition of seed, type of soil flora, sometimes flora of seed, environmental factors at time of seeding, and on the fungicide used. Ceresan and New Improved Ceresan were the most effective fungicides tested. These results help to explain the conflicting reports on the effects of seed treatments of flax.

Isolation of Bacterium angulatum from Overwintered Tobacco Field Soil. DIACHUN, STEPHEN, W. D. VALLEAU, AND E. M. JOHNSON. To determine whether *Bacterium angulatum* is present in overwintered soil in tobacco fields, samples of the soil were mixed with sterile water and poured onto the lower surface of susceptible tobacco leaves which were

artificially water-soaked just before inoculation. Water-soaking of the leaves permits bacteria present in water on the leaf surface to enter the leaf through stomata, and produce infection. Continued water-soaking is not necessary. Leaves were water-soaked by forcing a strong stream of water from a hypodermic syringe against the lower surface, the end of the needle of the syringe being held approximately one inch from the leaf. Between November 2, 1940, and April 18, 1941, *Bacterium angulatum* was recovered from field soil in which naturally infected tobacco had grown during the summer of 1940. One hundred eighty-two samples collected on 15 different dates were tested; *Bacterium angulatum* was isolated from 37 of these samples in 9 of the 15 tests.

The Reversal Effect: Why Fungicides Change in Rank in Repeated Field Tests. DIMOND, ALBERT E. The ranking of any fungicide may change radically in repeated tests. Many such changes may result from the reversal effect. If a series of dosages of each material is applied, a straight line relating fungicide dosage to disease control is obtained on logarithmic-probability coordinates. Under comparable conditions, the slope and LD95 of these curves are properties of the fungicide. When slopes for the dosage-control curves of 2 fungicides differ, these curves must cross one another. At the intersection point, the 2 materials have identical disease-controlling properties. Above this point, the material having the steepest slope is most effective; whereas, below this point, the material having the flattest slope is most effective by any method of comparison. Environmental changes influencing disease development will alter the slope of the dosage-control curve. From test to test, therefore, the point of intersection of dosage-control curves of the same 2 fungicides may be expected to change. If, in one test, the investigator compares his materials at a single dosage above the intersection point of their dosage-control curves, and, in a second test, compares them below this point, inversion in order of effectiveness of the materials can be explained.

Vacuolar Inclusions in Cells of Sugar Cane Affected with Chlorotic Streak. DUFRENOY, JEAN. Split stalks of sugar cane affected with chlorotic streak show red discolorations in the tracheary vessels, mostly at the nodes. In the parenchyma cells surrounding the vessels, spherical bodies having the structure of "Coacervates" are frequently observed. Surrounding each body is a membrane, apparently of phosphatides, as it yields molybdenum blue with molybdenum reagents and stains blue with nascent indophenol blue. The membrane encloses a dense colloidal mass rich in catechol and catechol oxidase, as demonstrated by the rapid oxidation of para- or ortho-phenols and the rapid absorption of neutral red, safranin, and other basic dyes used as vital stains. Coacervates, however, have also been observed in cane free of chlorotic streak and commonly in lesions of red rot (*Colletotrichum falcatum*). They are also common in the tissues of various other plants that are in a diseased or unthrifty condition. They have often in the past been mistaken for organisms of one type or another.

Sterilization of Rhizoctonia Sclerotia with Corrosive Sublimate. ELMER, O. H. A momentary dip of Rhizoctonia-infected seed pototoes in acidulated 3–500 concentration $HgCl_2$ sterilizes adhering sclerotia more effectively than does a 10-minute treatment in the concentration 1–500. Fifteen comparative tests since 1936 resulted in 74.7 per cent Rhizoctonia-free sprouts following the momentary dip and 64.2 per cent Rhizoctonia-free sprouts following the 10-minute treatment. Nontreated controls averaged 7.3 per cent Rhizoctonia-free sprouts. Soil-borne Rhizoctonia-infection frequencies were not greater than 3.8 per cent. Death of Rhizoctonia sclerotia resulting from the momentary or the 10-minute treatment evidently occurs following planting and not during the treatment period. After sclerotia-covered tubers were treated by the momentary or by the 10-minute treatments, then dried and cut into half, those half-tubers held 14 hours in running water to remove adhering $HgCl_2$ developed sprouts of which 89.1 and 91 per cent, respectively, were infected with Rhizoctonia. The unwashed half-tubers developed sprouts of which only 28 and 41.5 per cent, respectively, became infected. Effective Rhizoctonia control evidently requires a sufficient dosage of $HgCl_2$ on the planted tubers, either applied momentarily in high concentrations or during a soak treatment at lower concentrations. Momentary dipping in acidulated 1–500 $HgCl_2$ was ineffective.

Dormant Applications of Lime Sulphur for Controlling Raspberry Anthracnose. ELMER, O. H. Spring applications of commercial liquid lime sulphur to anthracnose-infected dormant black raspberry canes (6-year experiments) resulted in the following percentages of infection on subsequently produced new canes: anthracnose-free, 35.8; canes with occasional isolated lesions, 46.3; medium infection, 13.8; severe infection, 4.2. Infection percentages occurring on the new canes from nonsprayed controls were: anthracnose-free, 4.8; occasional isolated lesions, 10.3; medium infection, 31.7; severe infection, 57.6. Consecutive annual applications of only a dormant spray practically eliminated anthracnose from an experimental raspberry field. A November application of

1–10 lime sulphur to the canes was ineffective for controlling anthracnose. An application made just prior to the appearance of leaves was more effective than one made a month earlier. Lime sulphur is arrarently more lethal to the anthracnose organism in the old cane lesions when the peritheeia approach maturity than earlier in the dormant season, when they are still immature. Control is evidently due to prevention of sporulation through sterilization of old cane lesions and not to preventive applications on the new canes. Both Bordeaux 3–6–50, applied as a dormant spray, and lime sulphur 1–40, applied after leaves have appeared, were ineffective for preventing anthracnose.

Attenuation of Phymatotrichum omnivorum Cultures by Repeated Transfer of Young Mycelium. EZEKIEL, WALTER N. Successive transfer of cultures of *Phymatotrichum omnivorum* during the relatively early development of the colony causes rapid attenuation. There is extreme reduction in rate of growth, and, eventually, nearly complete failure of growth, making further transfer impossible. Attenuation has resulted from repeated weekly transfer from agar plates of peripheral discs (at 4 cm. from the original inoculum, and thus carrying mycelium only 1–2 days old) as compared to transfer of proximal discs (within 1 cm. of the inoculum, and thus carrying mycelium 4–5 days old). Proximal transfer lines retained somewhat vigorous growth, although inferior to that of lines transferred similarly but at 3-week intervals, while peripheral lines declined rapidly. Preliminary experiments indicate that the immediate cause of the attenuation is probably neither accumulation in attenuated cultures of diffusible inhibitory materials (as a phage) nor lack of the growth-promoting materials that have as yet been tested.

Cotton Root Rot, the Weather, and Cotton Yields. EZEKIEL, WALTER N. Within certain limits, increased rainfall during the several months preceding harvest leads to corresponding increase in the prevalence of root rot, caused by *Phymatotrichum omnivorum*, but additional rainfall during this period also favors growth of the cotton plant. Losses correctly attributable to root rot may be masked in average figures by increased yields from surviving plants and particularly the much higher yields in fields where the disease is not prevalent. For entire counties, average yields per acre, therefore, generally increase with greater prevalence of root rot, to a peak with around 25 per cent root rot. Above this, county-yield figures decline. For example, in Bell County in 1939, 10 per cent root-rot prevalence accompanied an average yield per acre of 135 lb.; 14 per cent in 1938, yield 157 lb.; 20 per cent in 1928, yield 184 lb.; and 29 per cent in 1937, yield 146 lb. Disastrous losses from root rot thus may not show from simple comparison of average yields and average prevalence. Several lines of work have instead furnished a basis for estimating percentage reduction in yield as approximating nine-tenths the percentage of plants killed by date of picking.

Tetrachloro-para-benzoquinone, an Effective Organic Seed Protectant. FELIX, E. L. Tetrachloro-para-benzoquinone has been tested further in the greenhouse as a seed protectant against damping-off under conditions of high soil moisture. In 41 tests in which Spergon, a commercial preparation containing 99 per cent tetrachloro-para-benzoquinone as the active ingredient, was applied to peas at a dosage of ¼ per cent of the seed weight, the mean difference in stand was 52.9 per cent and in height of plant 0.85 cm. in favor of the treated, with odds of over 1000 to 1 respectively, 10 days after planting. Active dosage of ⅛ per cent the seed weight in diluted and undiluted form yielded somewhat less stand than the higher dosage, but resulted in slightly taller plants in the diluted form. Addition of derris root, containing 5 per cent rotenone, to Spergon appeared not to reduce the effectiveness of the fungicide, indicating possible compatibility of the two. Spergon treatment of machine-delinted cotton seed at the rate of 3 oz. undiluted or 4 to 6 oz. of 25 per cent Spergon in talc per bushel of seed, effectively controlled damping-off in flats of Mississippi cotton soil.

Infection of Forage Grasses with Flag Smuts of Wheat, Rye, and Grasses (Urocystis tritici, U. occulta, and U. agropyri, respectively). FISCHER, GEORGE W. Inoculations of 32 species of *Agropyron, Elymus, Hordeum,* and *Sitanion* with smut spores of *Urocystis tritici, U. occulta,* and *U. agropyri* resulted in the development of typical flag-smut symptoms on the following grasses: *U. tritici* infected *Agropyron caninum* (2 accessions), *A. repens, A. spicatum* (2 accessions), *A. semicostatum, Elymus glaucus* (2 accessions), and *E. triticoides; Urocystis occulta* infected *Agropyron caninum, A. inerme,* and *Elymus canadensis* (3 accessions); *Urocystis agropyri* (taken from *Hordeum nodosum*) infected *Agropyron caninum* and *Elymus canadensis* (4 accessions). The 3 flag smuts seem to have entirely different host ranges among the grasses used. *Urocystis tritici* and *U. agropyri* are practically identical in their morphology, and it is recommended that they be considered as races of the same species, which, by priority, would bear the name *U. agropyri.* The spores of *U. occulta* seem to be less completely invested by the sterile cells, which character, if it proves to be constant, would distinguish the rye flag smut from

that of wheat and grasses. The demonstrated susceptibility of grasses to flag smut of wheat and the morphological identity of this smut with the similar flag smut on grasses suggests a possible explanation of the source of outbreaks of wheat flag smut in the U. S. where flag smut of grasses has long been known from coast to coast on a wide variety of grass species.

Comparative Value of Certain Popular Fungicidal Dusts in Control of Head Smut (Ustilago bullata) and in Improvement of Stands in Forage Grasses. FISCHER, GEORGE W. Copper carbonate and basic copper sulphate (about 50 per cent copper), formaldehyde dust (Formacide), Semesan (30 per cent Hydroxymerchlorophenol), "2 per cent Ceresan" (2 per cent ethyl mercury chloride), and New Improved Ceresan (5 per cent ethyl mercury phosphate) have been given comparative tests at Pullman, Washington, for control of head smut of grasses and for improvement of grass stands. The grasses used were *Agropyron trachycaulum* (slender wheatgrass), *Bromus marginatus* (mountain bromegrass), *B. catharticus* (rescue grass), *Elymus canadensis* (Canada wild rye), *E. glaucus* (blue wild rye), and *Hordeum nodosum* (meadow barley). The copper dusts were generally ineffective in controlling head smut, even at as high a rate as 6 oz. per bushel of seed, and improved stands only slightly. The same results were obtained with formaldehyde dust. Semesan, at 2, 4, or 6 oz. per bushel of seed, gave, generally, greatly improved stands, but very little smut control. In striking contrast to the poor smut control obtained with the above dusts, "2 per cent Ceresan" and New Improved Ceresan gave excellent smut control and from 300 to 500 per cent better stands than the untreated check rows. On the basis of these results, either "2 per cent Ceresan" at 2 to 4 oz. per bushel of seed or New Improved Ceresan at $\frac{1}{2}$ to 1 oz. per bushel can be recommended where combined head-smut control and excellent stands are desired. Except with *Hordeum nodosum*, the use of these dusts in considerable excess (2–4 times the recommended dosage) did not reduce the stands.

The Inheritance of Pathogenicity in a Cross between Physiologic Races 22 and 24 of Melampsora lini. FLOR, H. H. The pathogenicity of 133 F_2 cultures of a cross between physiologic races 22 and 24 of *Melampsora lini* as indicated by the reaction of 17 rust-differentiating flax varieties was determined. J.W.S., immune from both parent races, was immune from the 133 F_2 cultures. Buda, susceptible to both parent races, was susceptible to all F_2 cultures. Pathogenicity to Williston Golden and Williston Brown was inherited as a unit. These varieties, susceptible to both parent races, were resistant to 17 and susceptible to 116 of the F_2 cultures. Ability to produce a virulent infection type on all varieties, except Williston Golden and Williston Brown, was inherited as a recessive character. Pathogenicity to Bombay, Newland, Tammes' Pale Blue, and Ottawa 770 B was independently inherited and conditioned by single pairs of factors. Pathogenicity to Akmolinsk, Abyssinian, and C.I. No. 836 was inherited as a unit in a simple Mendelian ratio, as was pathogenicity to "Pale-blue crimped" and Kenya. Pathogenicity to Argentine and Bolley Golden, varieties having 2 pairs of factors for resistance to race 24, and to Italia Roma. C.I. 357–1 was conditioned by 2 pairs of factors. The 133 F_2 cultures yielded 68 physiologic races, 66 of which had not previously been isolated. (Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the North Dakota Agricultural Experiment Station.)

Identity of the "Intermediate" Mosaic in the Sugar-cane Variety C.P. 28–70. FORBES, I. L. AND P. J. MILLS. It has been reported previously that in the sugar-cane variety C.P. 28–70 the presence of a green mosaic virus would protect the plant against a yellow mosaic, and *vice versa*. Plants inoculated with mixed viruses would develop either the green or the yellow mosaic symptoms,—but not both. The results were of interest as bearing on immunity. Further inoculation tests with these viruses have revealed that an occasional plant will harbor both viruses and display their respective symptoms. In 5 experiments a total of 295 plants inoculated with juice from stalks showing mixed symptoms gave results as follows: 142 plants without symptoms, 84 with green mosaic, 32 with yellow mosaic, and 37 with mixed symptoms. With the same inoculation technique green- or yellow-mosaic material alone gave rise to the respective symptoms in about 80 per cent of the inoculations. The results suggest an antagonistic action between the two viruses, but, occasionally, they may occur together in the same plant. There is no evidence that the host produces any substance conferring "immunity."

Relation of the Hot-water Treatment of Sugar Cane to the Development of Red Rot. FORBES, I. L. Chlorotic Streak of sugar cane may be eliminated from seed cane by immersion in water at 52° C. for 20 minutes. As such a treatment may become advisable as a control measure, studies have been made during the past two years on the effect of such treatment on cane irrespective of this disease. These studies have included the relation of the treatment to the control of the red rot (*Colletotrichum falcatum*), which also

is generally distributed throughout the Cane Belt. Tests with varieties Co. 281 and C.P. 28–11, considered resistant to red rot, indicated that the hot-water treatment made these canes more susceptible. Tests with the variety C.P. 33–243, considered susceptible to red rot, indicated that the hot-water treatment made this variety still more susceptible. However, suspensions of spores of the red-rot fungus were not infective after the heat treatment. There was also very little red rot in stalks inoculated 1 to 2 days prior to the hot-water treatment, which indicates that spores disseminated at planting time were destroyed. In cane, given a heat treatment 3 days after inoculation, the fungus was not entirely killed, but its development seemed to have been arrested.

Some Factors Influencing Curly-top-virus Concentration in Sugar Beets. GIDDINGS, N. J. Curly-top-virus concentration is neither easily nor very accurately determined. The most satisfactory method yet found is to feed nonviruliferous leaf hoppers for a short period on the plants or extracts to be tested, transfer them to healthy young sugar-beet plants, and determine the relative percentage of plants that become infected. Living plants were used as virus sources in this work. Feeding periods of 3 to 9 hours were found most satisfactory. The shorter feeding periods gave best results if conducted at a temperature of about 110° F. Highly resistant beets were found to have a significantly lower virus concentration than susceptible ones. The less virulent strains of curly-top virus were present in significantly lower concentrations than the more virulent strains. Beets, infected for several months, showed significantly lower virus concentration than those infected for a few weeks; but it is possible that soil nitrogen may be a factor in this case.

The Fungicidal and Phytocidal Properties of Some Metallic Alkyl-dithiocarbamates. GOLDSWORTHY, M. C., E. L. GREEN, AND M. A. SMITH. Laboratory and field studies to determine the fungicidal and phytocidal properties of sodium, iron, lead, zinc, copper, silver, and mercury dimethyl, diethyl and dibutyl dithiocarbamates indicate that the dimethyl compounds, as a group, the more toxic to spores than those of the diethyl and dibutyl series. The soluble sodium salts were phytocidal, as were some of their metallic derivatives. The copper and mercury salts were unsafe to use on apple, peach, and bean. The lead salts caused no injury to these plants but the iron, zinc, and silver compounds caused some injury under certain conditions. Weathering appeared to change the iron and zinc compounds so that they injured peach leaves. Lead dimethyl dithiocarbamate is the most promising of the group tested. One season's test against apple scab in Maryland and Missouri indicated that iron and lead dimethyl dithiocarbamates could be safely applied to apple varieties and would control scab under the conditions prevailing in 1941. Peach scab and peach brown rot tests in Maryland showed that iron dimethyl dithiocarbamate would control these diseases, but that it may injure foliage and delay fruit maturity. It did not control cherry leaf spot in Maryland.

Compatibility of Diluent Dusting Materials with Copper Fungicides with and without Calcium Arsenate. GOODWIN, M. W., S. L. HOPPERSTEAD, AND K. J. KADOW. Samples of dusts, both with and without calcium arsenate, were prepared, using 48 different diluent materials, each with red cuprous oxide, tribasic copper sulphate and copper oxychloride. These were analyzed for pH, water-soluble Cu, and water-soluble As_2O_5. It is apparent that the pH of the mixture is a good indication of the amount of water-soluble copper liberated. There is a good correlation between pH and water-soluble copper, with the danger point about pH 5.5. Below this pH, amounts of water-soluble copper are liberated that could be injurious to plants. When calcium arsenate is present, no water-soluble copper is liberated because of the presence of free calcium, which, in every case tested, kept the pH of the mixture well above pH 5.5. Such is not true in the case of water-soluble arsenic. Different calcium arsenates produced varying results. As a general rule, when dealing with the same calcium arsenate, the lower the pH, the higher the water-soluble arsenic. Above pH 10.0, very little water-soluble arsenic is liberated. However, the character of the arsenate itself, rather than either the diluent or copper used, will determine the safety of the dust mixture. Limited studies with certain organic materials added to dust mixtures indicate that the above generalizations do not always hold.

Soil Fauna in Relation to the Pit Scab of Potatoes. GRANOVSKY, A. A., AND A. M. PETERSON. Further field studies of soil microfauna, habitually found in lesions of pit scab of potato (*Actinomyces scabies*) show it is not normally responsible for scab infection. About 50 per cent of pit-scab lesions were found infested with soil fauna, predominately mites, annelids, and nematodes. Other pits are free from animal life. Only a single record of what appears to be *Pnyxia scabiei* is known from the State in the past year. This insect is thought in other States to be responsible for pit scab. The high incidence of the disease and the scarcity of the insect requires a different interpretation

of the problem. The maggots of Mycetophilidae are rarely found in the pits. Those found, are either *Sciara pauciseta, S. nitidicollis* and species closely related to *Forcipomyia pilosa*. More frequently encountered are several minute species of Collembola, belonging to *Sminthuridae, Isotomidae* and *Entomobryidae*. The most common inhabitants of pit-scab lesions are mites—*Rhizoglyphus hyacinthi, Rh. phylloxerae* and species of *Macrochelus, Cheyletus* and *Uropodidae*. Most of these are scavengers or have predacious habits. Next in abundance are the young stages of annelid worms, which feed in the lesions and often completely clean the pits of decaying organic matter. Pit scab is the result of interaction of a physiologic race of *Actinomyces scabies*, secondary bacterial decay, and mycetophagous soil microfauna.

A Study of the Control of the Yellow Dwarf Disease of Potatoes. HANSING, E. D. Yellow dwarf can be most effectively and satisfactorily controlled in New York State by the use of potato varieties that escape infection under conditions favoring a moderate to high spread of the disease in the field. In several replicated experiments the varieties Arran Banner, Chippewa, Golden, Houma, Jubel, Katahdin, Sebago, and Warba had less than 2 per cent infected plants. The percentages of infected plants for Russet Burbank was 2 to 11, for Rural 3 to 29, and for Green Mountain 6 to 53. The percentages of infected plants for 20 U. S. D. A. potato seedling varieties ranged from 0 to 72. The incidence of yellow dwarf was partially reduced by such factors as location of the potato field; date of planting; use of shielding crops; roguing current-season infected plants; early harvesting; and the selection of potatoes for seed from the middle of the field. Application of dusts and spray, such as pyrethrum and celite, sulphur, or Bordeaux mixture, also reduced the amount of infection. Factors of little or no value in the control of the disease were: application of commercial fertilizer, roguing tuber-borne infected plants, and selection of large tubers for seed.

New Suscepts of the Potato Yellow-dwarf Virus. HANSING, E. D. The following species of plants belonging to 3 different families were found to be suscepts of the yellow-dwarf virus: Crassulaceae—*Kalanchoe diagremontiana,* Cruciferae—*Barbarea vulgaris* and *Capsella bursa-pastoris* and Leguminosae—*Medicago lupulina. K. diagremontiana* is an ornamental species introduced from Madagascar. The other 3 species are fairly common weeds in western New York, where epiphytotics of yellow dwarf occur. The following new varieties of known susceptible species also have been found to be suscepts of the virus: *Trifolium pratense* var. Mammoth Red and *Trifolium repens* var. English Wild White and var. Kent Wild White.

Biological Control of the Mealy Bug (Pseudococcus spp.). HARRAR, J. G., AND J. J. MCKELVEY, JR. A fungus, previously reported as a virulent, specific parasite of the mealy bug, has been investigated as a potential control of this greenhouse and orchard pest. Laboratory experiments under controlled temperature and moisture conditions were entirely successful in the destruction of mealy bug populations. Subsequently, similar experiments were carried out in greenhouses with equal success. Observations in apple orchards indicate possible high mortality of mealy bugs from natural infection and that the artificial addition of inoculum added may be beneficial. From data obtained, it is evident that the pathogen is rapid and positive in action under favorable conditions and will destroy mealy bugs in all stages of development (exclusive of the egg stage). Under unfavorable growth conditions, the fungus produces highly resistant compound sclerotia that may remain viable several months. These sclerotia are responsible for the persistance of the parasite in the field and greenhouse. The name *Endosclerotium pseudococcia* is suggested for the pathogen.

The Behavior of Endothia parasitica on Chestnut Trees in California. HARRIS, M. R. Three chestnut orchards, located near Stockton, San Joaquin County, California, have been found infected by the chestnut blight fungus (*Endothia parasitica*). Two of the orchards have been observed since 1934 and a third since 1938. The disease is not known to exist elsewhere in the State, although chestnuts are widely planted both as shade and orchard trees. The origin of the disease is not known, but the fungus probably was brought in on cions secured in Eastern States. Observations on every infected tree found over a period of years failed to show any evidence of the perfect stage of the fungus, but pycnospores have been noted on a number of the trees. Lesions of the fungus have been found less often on the limbs and trunks above ground line as at ground line or below it. Contrary to the experience with the disease in Eastern States, the fungus has been very largely spread by pycnospores carried by irrigation water. There is no evidence of spread by air, birds, or splashing rain. A few trees have been infected by contaminated pruning tools. Control of the disease is being carried on by inspecting the orchards twice a year and destroying infected trees. Present indications are that it may eventually be eradicated.

The Relation of Vitamin B_1 to Crown-gall Development. HENRY, BERCH W., A. J. RIKER, AND B. M. DUGGAR. Some relations of vitamin B_1 to crown-gall development on tomato have been studied with the Phycomycees assay. Vitamin B_1 accumulated in almost maximum concentration at inoculation points within 1 week after treatment. The B_1 concentration in galls remained fairly constant from 3 to 5 weeks after inoculation—during the period of rapid increase in size. The B_1 concentration in galls, approached that in the growing tip of the host plant, was somewhat greater than that in mature leaves, and was much greater than that in mature stems of either inoculated or control plants. Temperatures above and below the maximum for gall formation had no effect on the B_1 concentration in inoculated plants. Galls produced by a partly attenuated culture of *Phytomonas tumefaciens* contained as high a concentration of B_1 as did the galls produced by the virulent culture. The bacterial cells of the partly attenuated culture contained as much vitamin B_1 as the cells of the virulent culture. Thus, vitamin B_1 may help in the initiation of crown gall, but it does not seem to have a causal role in gall development beyond that of any necessary food or growth factor.

Improved Control of Alternaria solani (Early Blight) on Tomatoes by Controlling Flea Beetles. HEUBERGER, JOHN W. An experiment was designed to determine the effect of derris on the protective value of several copper compounds (Bordeaux, tribasic copper sulphate, copper oxychloride, yellow cuprous oxide, and red cuprous oxide). Six applications were made (June 30–Aug. 21). Flea beetles were abundant during June and July. Records on July 28 and Aug. 8 on corresponding plots receiving copper alone and copper-derris, as spray or dust, showed that the copper-derris plots had fewer beetle-feeding punctures, fewer blight lesions, and much less defoliation than the copper plots. Derris, by controlling beetles, improved blight control in two ways: (1) reduced the number of wounds (feeding punctures), which serve as infection courts, and (2) reduced dissemination of *Alternaria* spores. On Sept. 8 the copper-derris plots still had less defoliation, even though beetles disappeared by Aug. 1. Thus, the deleterious action of derris on copper effectiveness, recently reported, apparently was overbalanced by the beneficial effect of beetle control earlier in the season. The following schedule should give effective blight control in areas where beetles are present early: copper-derris while beetles are present, followed by copper alone at 10–14-day intervals after the beetles disappear.

Effect of Copper Content, Completeness of Admixture of Copper and Diluent, and Nature of Diluent on Field Performance of Copper Dusts. HEUBERGER, JOHN W. Copper compounds tested were copper oxychloride and red and yellow cuprous oxide; dust diluents were Pyrax ABB, Eastern Magnesia #23 Tale, Loomkill Tale, Bancroft Clay. The dust materials were tested also as sprays to eliminate such factors as fractionation in duster, flowability, denseness of dust cloud, settling rate, etc. Materials were tested on tomatoes for control of *Alternaria solani*, using a 6-application schedule (June 30–Aug. 21). Metallic copper content was 1.5–100 for sprays and 7 per cent for dusts. Using hand equipment, both were applied in similar fashion to deposit 3 lb. metallic copper per acre per application. Increasing copper content from 4 to 8 per cent increased control from 18 to 36 per cent; improving completeness of admixture of copper diluent in an attrition mill increased control from 16 per cent for original sample to 33 per cent; the nature of the diluent influenced effectiveness of copper *e.g.*, descending order of control with same copper, as sprays, was Bancroft Clay, Loomkill Tale, Eastern Magnesia Tale, Pyrax ABB, while the order, as dusts, was Loomkill Tale, Bancroft Clay, Eastern Magnesia Tale, Pyrax ABB. When applied in same fashion, similar materials used as sprays and dusts gave approximately equivalent control.

Transmission of Pierce's Disease of Grapevines with a Leaf Hopper. HEWITT, WM. B., N. W. FRAZIER, AND BYRON R. HOUSTON. The natural spread of Pierce's disease of grapevines indicates an insect vector. In 1939, 54 species of insects collected from vineyards, alfalfa, and natural cover were tested in a field plot as possible vectors. Ten of the 94 vines upon which one or more species of insects were caged and only one of 215 control plants developed Pierce's disease. Sixty insect species were similarly tested in 1940. Nine of these test plants developed disease, 6 out of 21 caged with leaf hoppers of the genus *Draeculacephala* and 3 out of 19 with the genus *Carneocephala*, and only 6 of the 506 control plants developed disease. Leaf hoppers of the genus *Draeculacephala* were further tested in 1941 under controlled insectary conditions. To date, 9 vines in these tests show leaf scorching and cane immaturity, typical fall symptoms of Pierce's disease. Field observations show a close correlation between the incidence of Pierce's disease and alfalfa dwarf. Insects that transmitted Pierce's disease in 1940, though fed on diseased grapes, had been collected from alfalfa fields known to have dwarf. In the 1941 tests the leaf hoppers that apparently transmitted the virus had been fed on alfalfa dwarf plants.

Host Response of Maize Seedlings to Pythium graminicolum. HO, WEN-CHUN, AND JAMES M. KOEPPER. During the past 3 years open-pollinated varieties, inbreds, and single and double crosses of maize, grown in the greenhouse and field, varied widely in their response to *Pythium graminicolum.* In 14 varieties studied, Kossuth County Reliance and Stern Yellow Dent were most susceptible; Black Yellow Dent and Krug were resistant. Although the majority of the 15 inbreds tested were susceptible, Ldg (K), Black 349, Lancaster 289, Osterland 426 and Hy showed some resistance. Combinations of these less susceptible inbreds, in general, were the most resistant of all single crosses tested; Iodent 205 × Black 345 and Iodent 205 × Iodent 234 were very susceptible. The Iowa hybrids, Iowa 13, Iowa 931, and Iowa 939, seemed the most resistant of the 23 double crosses examined. The injury caused by *P. graminicolum* in combination with other pathogens was studied. Combinations with *Rhizoctonia solani, Helminthosporium sativum,* or *Diplodia zeae* increased the amount of injury. Combination with *Penicillium oxalicum* showed the same disease severity as *Pythium graminicolum* alone, but combinations with *Aspergillus niger* or *Trichoderma lignorum* caused less injury than *Pythium graminicolum* alone. In general, the symptoms from *P. graminicolum* and those from the active pathogens were distinct, but the destructive effect was additive. In combination with weak pathogens, symptoms of *P. graminicolum* prevailed, but the destructive effect was subtractive.

The Sorghum Root-and-stalk-rot Complex in Oklahoma. HOFFMASTER, DONALD E. Since 1939, a serious root-and-stalk-rot complex of sorghums has been under observation in Oklahoma. The disease complex may first appear when the plants are 3 or 4 inches tall, resulting in a seedling blight, and may continue to attack the surviving plants during the growing season, resulting in stunted plants with small, poorly developed heads. The symptoms of this disease complex closely resemble those of the Milo disease, but repeated isolations have failed to yield *Pythium arrhenomanes.* Instead, isolates of *Fusarium,* Bacteria, *Sclerotium bataticola, Helminthosporium* and *Pythium* (listed in order of prevalence) have been secured. Greenhouse and laboratory tests indicate that *Pythium* (not *arrhenomanes*) and *Sclerotium bataticola* are capable of causing seedling blight. The ability of *Sclerotium bataticola* to cause seedling blight is of especial interest because of the frequent occurrence of the fungus in stems of mature plants affected with charcoal rot. This is a disease frequently observed in plants subject to adverse environmental conditions.

Transgressive Inheritance of Pathogenicity Factors in Hybrids between Two Races of Tilletia tritici. HOLTON, C. S. Evidence of transgressive inheritance of factors for pathogenicity has been obtained from studies with hybrids between races 8 and 9 of *Tilletia tritici.* These races differ in that Hussar is susceptible to T-8 and resistant to T-9, while Hohenheimer is resistant to T-8 and susceptible to T-9. Hussar × Hohenheimer selection, C.I. 10068–1, is highly resistant to both of these races and all other known races of the bunt fungi, while Hybrid 128 is susceptible to all races. However, 4 hybrids between T-8 and T-9 prove capable of infecting all of these varieties and one other hybrid was pathogenic to Hybrid 128 only. The infection percentages on C.I. 10068–1 ranged from 29 to 56, thus indicating a rather high degree of susceptibility to the race hybrids. Furthermore those hybrids capable of infecting all of the above varieties were more virulent on Hohenheimer than T-9 and less virulent on Hussar than T-8. Apparently, therefore, in these hybrids between T-8 and T-9, transgressive inheritance of pathogenicity factors resulted in the production of biotypes possessing pathogenic properties, not only distinctly different from those of the parent races, but different from those of any other known race. (Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.)

The Effects of Mustard Oils on Germination of the Resting Spores of Plasmodiophora brassicae. HOOKER, W. J. The effects of two mustard oils (allyl isothiocyanate and beta phenyl ethyl isothiocyanate) upon spore germination of *P. brassicae* were studied under laboratory conditions with partly purified suspensions of spores and various concentrations of oil. The extent of germination was determined by counting the number of zoospores within a field of known volume at daily intervals. Although the exact concentrations at which germination was prevented varied with different spore lots, both oils were consistently effective at 80 p.p.m. and sometimes at as low concentrations as 10 p.p.m. of allyl isothiocyanate and 5 p.p.m. of beta phenyl ethyl isothiocyanate. Concentrations of both oils below the toxic level were found capable of definitely stimulating spore germination. This occurred in each of the 8 experiments carried out with allyl isothiocyanate and with 2 of the 5 experiments involving the phenyl ethyl oil. Stimulation was greater in the 2 cases with phenyl ethyl isothiocyanate than in similar concentrations of the allyl oil prepared and observed at the same time. These observations suggest that a toxic sub-

stance present in the host may have a stimulatory action upon the pathogen at certain low concentrations.

Comparing Fungicides at Dosages for Equal Control. HORSFALL, JAMES G., AND ALBERT E. DIAMOND. While making a quantitative study of fungicide performance, it developed that comparison through (1) dosage-for-equal-control was more sensitive and more informative than (2) control-for-equal-dosage. Dosage for equal control can always be obtained by interpolation or extrapolation on the straight line obtained from the logarithmic-probability plotting of data. Control for equal dosage has a low order of sensitivity because the control scale is limited by a ceiling of 100 per cent, whereas the dosage scale is unlimited. Red and yellow copper oxide dusts applied to muskmelons against *Macrosporium cucumerinum* in 1941 are useful illustrations because the slopes of the curves are parallel. The ratio between the two on the control scale for equal dosages of 9 per cent metallic copper in the dust was 1.12, but this ratio was magnified to 1.77 on the dosage scale for equal control of 80 per cent. A more serious limitation of (2) is that the ratio in performance of the two materials changes along the control scale, rising to 1.24 with equal dosages of 4 per cent. Finally (*vide* Diamond accompanying abstract) the occasional inversion in rankings of materials may be detected in the data obtained in making a comparison at dosage for equal control.

Leaf-hopper Transmission of the Alfalfa Dwarf Virus. HOUSTON, BYRON R., N. W. FRAZIER, AND WM. B. HEWITT. The dwarf disease of alfalfa has recently been found prevalent in the southern portion of the San Joaquin Valley, California, where it is now an important factor in the thinning of alfalfa stands. In 1940 healthy plants growing in large cages infested by mixed species of insects collected in diseased fields resulted in 63 per cent dwarf diseased plants. Transmission tests also were made with individual species of plant-feeding insects collected in diseased fields. The only cases of transmission were in 2 of 3 tests with a leaf hopper of the genus *Draeculacephala*. A later trial with 100 individuals of this species caged on dwarf plants and subsequently transferred to 30 healthy plants resulted in 93 per cent disease. The virus was then transmitted from these diseased plants to healthy plants by grafting. Nine transmissions were later obtained with leafhoppers of the genus *Carneocephala*. In the summer of 1941, insectary inoculations were made involving several species of insects and several hundred alfalfa plants. To date 19 transmissions of alfalfa dwarf have been obtained with leaf hoppers of the genus *Draeculacephala* and no control plants have shown symptoms of the disease.

The Effect of Various Organic and Inorganic Compounds on the Growth of Sclerotinia sclerotiorum. HOYMAN, WM. G. Forty-four organic and inorganic compounds were tested in the laboratory to determine which would inhibit the growth of *Sclerotinia sclerotiorum*. When soluble, each compound was added to the medium in concentrations of 1: 1,000, 1: 10,000 and 1: 100,000. Lest certain transformations and losses might result from autoclaving the chemicals employed in this experiment were not thus treated. Malachite green, 3,5-dinitro-o-cresol, mercuric chloride, and 2,4-dintrophenol inhibited the growth of the organism at the lowest concentration (1: 100,000). These 4 chemicals were tested further at concentrations of 1: 200,000, 1: 400,000 and 1: 800,000 in order to determine which was the most toxic. Malachite green and 3,5-dinitro-o-cresol proved to be the most effective in inhibiting the growth of *Sclerotinia sclerotiorum*.

Breeding Cantaloupes for Resistance to Downy Mildew and Other Diseases and Pests. IVANOFF, S. S. Some progress has been made in breeding shipping-type cantaloupes for resistance, mainly to the downy mildew (*Peronoplasmopara cubensis*). Sources of resistance to downy mildew have been selected inbreds derived from the varieties Cuban Castillian and Rocky Dew (green flesh). Sources for commercial shipping qualities have been the varieties Arizona Nugget, Seed Breeders, and Powdery Mildew Resistant California 45. Some cantaloupe lines thus far obtained in the F_4, F_5 and F_6 generations have shown shipping qualities combined with varied resistance to downy mildew, powdery mildew (*Erysiphe cichoracearum*), the melon aphid (*Aphis gossypii*), and to damage caused on the leaves by larvae of *Diaphania* spp.

The Nature of Eggplant "Yellows." IVANOFF, S. S. This serious virosis of eggplants in South Texas, characterized by mottling, spotting, and yellowing of the leaves, has been found to be sap-transmissible. Its natural vector is still unknown. The disease has been transmitted to cucumber by rubbing with infectious juice, producing leaf mottling. It has been induced on eggplant by rubbing with juice obtained from cantaloupe plants grown in the field and exhibiting a type of mosaic symptoms. Efforts to transmit the disease from eggplants to tomatoes, peppers, potato, and other plants have thus far failed. Transmission from eggplant to eggplant has been accomplished also by grafting and by needle puncturing the stem through a drop of plant sap containing the

virus. The incubation period varies from 8 to 21 days, apparently depending on the season, rate of plant growth, and source of inoculum. The infectivity of the virus seems to be seriously affected by drying. The virus appears to be closely related to the cucumber group of viruses.

A New Species of Elsinoë on Capulín Cherry (Prunus capuli). JENKINS, ANNA E. A new species of *Elsinoë* is described. This produces bright leaf spots on capulín cherry, closely related to wild black cherry (*Prunus serotina*). The type locality of the *Elsinoë* is the nursery of the Agricultural Experiment Station, Caracas, Venezuela, where the fungus was discovered in January, 1940, by M. F. Barrus and A. S. Müller, who forwarded specimens to the writer for study. The infected tree had been brought from Mexico. According to the literature Mexico is the country of origin of this much esteemed fruit tree, and the cherry was mentioned by the earliest Spanish visitors to the country. The perfect stage of the *Elsinoë* is present in abundance on the specimen mentioned, and the imperfect stage (*Sphaceloma*), on a subsequent gathering (March, 1941) by Müller. The existence of *Sphaceloma* on plum (*P. domestica*) was shown by the writer in 1932, through Garbowski's report of *Hadrotrichum populi* on this host growing on the peninsula Crimea (South Russia), or in its vicinity. Specimens are not available, however, as was learned from correspondence with Garbowski a number of years ago. During the past few years a hitherto unreported *Sphaceloma* on choke cherry (*P. virginiana*) was discovered in Canada and the United States by A. A. Bitancourt and the writer.

The Influence of Temperature and Moisture on the Development of the Intermediate Loose Smut of Barley. JOSEPHSON, L. M. Controlled experiments were conducted in the greenhouse at Madison, Wisconsin, to determine (1) the effect of constant soil temperature and soil moisture on the development of smut, and (2) the effect of change of soil temperature during the growth of the host on the incidence of barley smut caused by *Ustilago medians* (*U. nigra*). Soil temperatures of 5° and 30° C. were generally unfavorable, 10° and 25° C. more favorable, and 15° and 20° C. optimum for smut development. Air temperature did not appear to influence smut development when soil temperature was constant. Soil moisture did not give consistent results as a factor influencing smut development. Changes from the low to the optimum or high soil temperature after plant emergence resulted in more smut than maintenance of constant low temperature during the growth of the plants. Similar results occurred in the change from the high to the optimum soil temperature. However, changes from high to low and from optimum to either high or low soil temperatures decreased smut. The effect of temperature changes after the plants had reached the second or third leaf-stage was less pronounced.

Physiologic Races in the Fungus Causing the Intermediate Loose Smut of Barley. JOSEPHSON, L. M. Approximately 100 collections of *Ustilago medians* (*U. nigra*) from the North Central States were studied for their pathogenicity on 17 varieties of spring barley, critical tests having been made on 9 varieties. By tests covering a period of 5 years, 8 physiologic races of the fungus were differentiated. These races can be differentiated on the basis of their pathogenicity on the following varieties: Excelsior, C.I. 1248; Himalaya, C.I. 2448; Manchuria, O.A.C. 21, C.I. 1470; Wisconsin Barbless, C.I. 5105; Lion, C.I. 923; and Hannchen, C.I. 531. Odessa, C.I. 934, is susceptible to all 8 races. Race 2 was found in Minnesota only, races 4 and 5 in North Dakota only, race 7 in Illinois only, while races 1, 3, and 8 were widely distributed in the States from which collections were obtained. Race 6 was obtained from New York State, from which no other collections were received. Races 3, 4, and 5 are classified on the basis of small differences in pathogenicity, which make it desirable to further check these races under different environmental conditions.

The Compatibility of Fruit-drop Sprays and Other Common Spray Materials. KADOW K. J., AND S. L. HOPPERSTEAD. Sprays to prevent the premature dropping of apples are rapidly gaining general usage. When used on early varieties, it is often desirable to apply them along with other spray materials. Naphthaleneacetic acid (Parmone) was applied to Williams at 5 p.p.m. and to Delicious and McIntosh at 10 p.p.m. in combination with Black leaf 155, 3 lb.; Phenothiazine (Micronized), 2 lb.; Genicide, 1½ lb. + Genifilm A, 3 oz. + kerosene, 1½ pt. + Genifilm B, 2 oz.; Bordeaux 1–3–100 + lead arsenate, 2 lb.; lime, 20 lb. + aluminum sulphate, 3 lb.; and ground Derris (5% Rotenone), 3 lb. + Grasselli spreader sticker, 3 oz. In addition to the above combinations, the sodium salt of naphthaleneacetic acid (App-L-Set) was used at ¼ lb. with and without aluminum sulphate, 3 lb. + lime, 20 lb. Likewise, Fruitone, which is naphthaleneacetic acid + an amide of it, was also used (½ lb.) with and without aluminum sulphate, 3 lb. + lime, 20 lb. The results obtained indicate that naphthaleneacetic acid is compatible with all combinations except those containing lime. When lime was added the effectiveness of all three fruit-drop materials was reduced from 25 to 50 per cent. Lime, used as a spray ingredient earlier in the season, did not reduce the effectiveness of fruit-drop sprays, applied to the same

trees later on. (Cooperative study with Grasselli Chemical Department, E. I. du Pont de Nemours Co., Wilmington, Delaware.)

The Production of Spores of Diplodia zeae in Culture. KENT, G. C. The production and germination of the spores of *Diplodia zeae* formed on the host or in culture has been found to vary greatly with the environmental conditions. The production in culture of spores of the most uniformly high germinability was secured by growing *Diplodia zeae* on oatmeal-extract agar solidified in a thin layer over the sides and bottom of an Erlenmeyer flask. The inoculated flask was incubated in a moist chamber and exposed to light and to a temperature of 20° C. After 6–10 weeks spores so produced were suspended in distilled water, washed twice and then suspended in the germinating medium, 2 per cent dextrose, 1 per cent starch, and carrot decoction being equally favorable under most conditions. Germination under the stated conditions occurred in 13–16 hours and was equally high, 95–100 per cent, whether or not the drop was covered by a cover slip. The percentage germination was lower if the spores were from cultures less than 6 weeks or more than 10 weeks of age.

Nitrogen Sources Utilized by Some Bacterial Plant Pathogens. KENT, G. C. The sources of nitrogen utilized by the peritrichous bacterial plant pathogens were studied as a possible adjunct to their classification. A test of usage was considered as positive only if the bacteria survived after 5 successive transfers at 48-hour intervals in liquid medium containing nitrogen in the test substance only. By this criterion, and with glycerol as a carbohydrate source, it was found that cultures designated as *Bacillus carotovorus, B. phytophthorus,* and *B. aroidae* could utilize nitrogen in the organic or inorganic form, whereas *B. amylovorus, B. tracheiphilus,* and *B. salicis* could utilize only complex organic forms of nitrogen. Of 2 cultures designated as *B. lathyri* one could utilize nitrogen as nitrate or ammonia, whereas the other could utilize only ammonia-nitrogen, in addition to the organic-nitrogen forms.

Reaction of Varieties and Selections of Oats to Pseudomonas coronafaciens. KINGSOLVER, C. H. Epiphytotics of halo blight occurred in Iowa in 1940 and 1941. The varieties Hancock, Marion, and Boone were more heavily infected than Fulghum, Gopher, Columbia, and Albion. Data obtained from breeding nurseries at Ames and Kanawha, Iowa, confirmed these field reactions. Most selections from the cross Markton × Rainbow were heavily infected. Selections of Victoria × Richland showed moderate amounts of infection; a few were only slightly infected. Most selections of Anthony × Bond showed moderate amounts of infection, however, the range was greater than in the Victoria × Richland selections. Almost all selections of D69 × Bond were outstanding in their relative freedom from infection. This was of interest because selections from this cross were resistant to most races of both rusts and both smuts. Certain of these selections will be increased in 1942 for possible distribution to Iowa farmers. Most selections from crosses involving Mutica Ukraina, (Victoria × Richland) × Columbia, Gopher × Boone and (Bond × Anthony) × Boone showed very heavy infection. Selections of (Iogold × Bond) × Boone showed consistently a moderate amount of infection. In *Avena byzantina,* Bond, Fulghum, and Victoria were outstanding in their freedom from infection. Preliminary greenhouse trials have substantiated field data. (Cooperative investigations of Botany and Plant Pathology Section, Iowa Agricultural Experiment Station, and Bureau of Plant Industry, U. S. Departmen tof Agriculture.)

Resistance in South American Lycopersicon Species to Early Blight and Septoria Blight. LOCKE, S. B. Application of a laboratory test to approximately 50 selections embracing 5 South American *Lycopersicon* species revealed in *L. hirsutum* resistance to *Septoria lycopersici* and *Alternaria solani,* the causal agents of Septoria blight and early blight, respectively. The laboratory test also showed the F_1 hybrids of the cross *L. esculentum* × *L. hirsutum* and the resistant parent equally resistant to Septoria blight, but intermediate with respect to early blight. Defoliation and leaf-spotting data, obtained in the field in 1941 under severe attacks by *Septoria lycopersici,* are in agreement with those from the laboratory tests. A field test for early-blight resistance was not obtained. Field data also indicated an approximate 1 to 1 segregation in the first backcross to the susceptible parent with respect to Septoria-blight resistance. While these data are not enough to establish the mode of inheritance, they do suggest that resistance to Septoria blight may be associated with a single, dominant genetic factor.

Biotypes within Puccinia graminis tritici, Race 15. LOEGERING, W. Q., AND E. C. STAKMAN. It has been known for some time that there may be biotypes within some physiologic races of *Puccinia graminis tritici.* Race 15 appeared especially suitable for study because collections of this race have fallen into two groups, designated for convenience 15A and 15B. Race 15A has been found in Japan and the United States. Race

15B has been found commonly in South America and is not uncommon in the United States. The latter is somewhat more pathogenic to some of the differentials than the former. Inoculations were made with representatives of both groups on several rust-resistant varieties of wheat and emmer. Rival, a newly recommended rust-resistant spring wheat, is resistant to 15A but completely susceptible to 15B, both in the seedling and adult-plant stages; hence it can be used as a differential. It is probable that the addition of other new varieties to the group of differentials may make possible a finer analysis of other physiologic races also. Practically, it is important, in testing for resistance, to inoculate new varieties with representative collections of races unless their composition is well known. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

The Response of Some Field Crops on Soils Treated with Chlorpicrin. MCLAUGHLIN, J. HARVEY, AND I. E. MELHUS. Duplicate soil-fumigation experiments were conducted on black, heavy Clarion loam and on light, sandy Buckner soil. These soils were treated with chlorpicrin in September, 1940, at the rate of 480 lb. per acre. Crops planted were oats, wheat, flax, barley, alfalfa, and sugar beets. Seedling-stand counts showed important differences in favor of treated over untreated soil. Isolations from seedling roots yielded fewer pathogens from treated than from untreated soil. The green and dry weights of the harvested crops showed pronounced differences in favor of treated over untreated soil with one exception: a planting of alfalfa on Clarion loam soil. Grain yields for the small grains and flax grown in treated soil varied from slightly greater to 5 times as great as the yield for those grown in untreated soil. The sugar beets grown in treated soil were 3 to 10 times the size of the beets in the untreated soil.

Witches' Broom of Alfalfa in Washington. MENZIES, J. D., AND F. D. HEALD. A diseased condition of alfalfa plants, characterized by the production of an abnormally large number of dwarfed, spindly shoots, has been reported occasionally in the northwestern States since 1924. It appears to be very similar to the destructive witches' broom virosis of alfalfa in Australia, but, in this country, has been considered to be of minor importance. Within the last 5 years, however, witches' broom has become epiphytotic in the Methow Valley of Washington, rendering 3- and 4-year-old alfalfa stands unproductive; in some cases it has completely discouraged reseeding to alfalfa. It is not uncommon to find fields wherein over 70 per cent of the plants are affected. Data suggest that this disease is due to a virus. Transmission attempts were made in 1940–41 using mechanical methods, grafting, and possible insect vectors. Limited variety trials show that the wilt-resistant varieties Ladak, Turkistan, and Hardistan are among the most susceptible to witches' broom, and no variety tested has shown marked resistance.

Physiologic Races of Ustilago tritici. MOORE, M. B. In investigations covering 6 years, at least 5 distinct physiologic races of *Ustilago tritici* have been distinguished by the relative ability of collections from different sources to cause infection on certain bread wheats and durums. In general, collections cause abundant smut in only one or the other group of wheats, although some can attack certain varieties in both groups. Most field collections have behaved like single races and have remained relatively constant over a period of years. In at least one case, however, there was a tendency for the virulence of a collection to decrease after successive annual inoculations on the same variety. When certain races were mixed, it was possible to separate them again by inoculating appropriate differential varieties, even after the mixture had been grown 1 year on a host equally susceptible to the component races. What was apparently the same race has sometimes been obtained from geographic regions as widely separated as Texas and the spring wheat region of Minnesota and North Dakota.

Studies on the Performance of Fungicide Diluents, Their Base Exchange and Copper-adsorption Capacities. NIKITIN, A. A., AND E. G. ANDERSON. It was found that commercially available dust diluents vary in their chemical composition, base exchange capacity, and adsorption properties. The term "inert," adopted for these materials, is misleading, since actually they have a great influence upon the performance of the copper fungicide. The difference in their behavior toward the copper ion is due to the difference in their base exchange capacity and to the variation in their physical properties, such as adsorption. The variation in the base exchange and copper adsorption capacities of the dust ingredients is of particular interest. The adsorption capacity of tales for copper approaches that of limestone, while that of clays is much less than that of tales. The buffering action of diluents possessing high adsorption capacities for copper may be beneficial to crops most sensitive to copper injury. Less adsorptive materials may be suitable on resistant plants. Adsorption properties of diluents also may protect against leaching of soluble copper by rainfall. Spreaders, such as wheat flour, soya flour, or bentonite,

change the adsorption capacity of the diluents. It was found that the adsorption capacity of talc was reduced by bentonite or flour.

Pathogenicity Studies of Rhizoctonia Isolates on Potatoes and Beans. PERSON, L. H. Rhizoctonia cultures were isolated from single sclerotia from potatoes from Nebraska, North Dakota, Maine, and Colorado. From 65 of these, tested on potato stems in sterilized soil, 23 were non-pathogenic, 27 only slightly pathogenic (producing only light, superficial lesions), 13 moderately pathogenic, and only 2 produced severe lesions. Two isolates from sugar beets and 2 from bean stems produced severe lesions on potato stems, while one isolate from bean and one from cauliflower produced moderate lesions. Of the 65 potato isolates tested on bean seedlings, 54 were non-pathogenic, 9 produced only slight superficial infection centers on 16 out of 267 seedlings, and 2 were only slightly pathogenic, producing small but definite lesions. The bean, sugar-beet, and cauliflower isolates produced moderate to severe lesions on the bean seedlings. In previous work (unpublished) 11 potato isolates in 6 different tests were found non-pathogenic on bean seedlings. This indicates that Rhizoctonia isolates obtained from potato tubers are not pathogenic on bean seedlings, and that lesions associated with potato stems are probably to a greater extent caused by soil-borne Rhizoctonia than by Rhizoctonia developing from the sclerotia borne on the seed potatoes.

Physical and Chemical Adaptation and Environmental "Carry Over" Effects in Ustilago zeae. PETTY, MILTON A. A constant haploid monosporidial line of *Ustilago zeae* was grown for 7 continuous cultural generations at about 21° C., at 32° C., and 35° C. Duplicates were exchanged among the temperatures. At 32° C. and at 35° C. there arose 13 mutants, 1 temporary variant that persisted for 2 generations, and 2 variants that did not persist beyond the generation of their origin. The constant and a variable haploid monosporidial line were grown as many as 9 cultural generations on synthetic media containing sodium arsenite, mercuric, ferric, and sodium chlorides whose concentrations were usually increased each generation. Cultures thus gradually adapted grew on a 0.558 per cent arsenic medium, but unadapted cultures failed to grow. Mutations occurred frequently on arsenic media in both lines. No definite results were obtained with either mercury or iron media, as the results were quite variable from generation to generation. Both lines of *U. zeae* grew faster after 3 generations on a 10.8 per cent sodium chloride medium than did first-generation cultures. This was apparently an osmotic adaptation. In conclusion, acceptable scientific proof was not obtained because the possibility of mutation could not be eliminated in the experiments.

The Reaction of Cantaloup Strains to Powdery Mildew in the Greenhouse and in the Field. PRYOR, DEAN E., AND THOMAS W. WHITAKER. Symptoms of cantaloupe powdery mildew were separated into 5 classes. Utilizing this classification, the problem of comparing symptoms in the greenhouse with those occurring in the field has been approached by two methods. (1) A comparison has been made of the symptoms in strains of cantaloupes artificially inoculated in the greenhouse with those of plants exposed to natural field infection. Of the 18 strains tested in the greenhouse, all except one highly resistant strain showed a 4 reaction (most severe). Infection on these same strains in the field varied with the planting date, being more severe in the last two plantings than in the first one. Except for the last planting, in which all the tolerant and susceptible strains gave a type-4 reaction, a majority of the tolerant strains were superior to the susceptible check. (2) Records of individual plants tested in the greenhouse, then transplanted to the field, indicate that if all macroscopic symptoms be taken into account, susceptible plants can be eliminated dependably in the greenhouse. These experiments have served as a basis for the development of a greenhouse technique that provides an accurate and practical method of judging the mildew resistance of cantaloupe without the time or expense involved in field trials.

The Influence of Vitamin B_1 on the Development of Cantaloupe Powdery Mildew. PRYOR, DEAN E. Of the two melon strains employed, the susceptible variety was readily attacked by race 2 of *Erysiphe cichoracearum*, while the resistant line developed necrotic spots following severe artificial inoculation. When thiamin chloride in various concentrations was added to soil in which diseased plants were growing, the number of colonies on the susceptible melons was increased 30 to 50 per cent, and necrosis on the resistant strain 110 to 180 per cent over the controls. The differences were statistically significant. When leaves from mildew-free susceptible plants were excised, inoculated, and maintained on a sucrose solution to which various amounts of thiamin were added, non-significant increases in mildew resulted. When leaves from mildew-free plants, grown in thiamin-treated soil, were excised, inoculated and maintained on the sucrose solution alone, those from plants previously watered with .01 p.p.m., thiamin solution produced barely significantly more mildew growth.

Experiments on the Yellows Disease of Sour Cherry (Prunus cerasus). RASMUSSEN, E. J., AND DONALD CATION. Cherry yellows was transmitted by budding from cherry to cherry, cherry to peach, peach to peach, and peach to cherry. Inoculations in 1939 on 6-year-old Montmorency cherry trees resulted in symptoms 2 years later, while 1940 inoculation of spring-planted Montmorency year-old nursery trees produced symptoms the following year. Control trees showed no symptoms. Three strains of cherry yellows have been differentiated by reactions on peach and Mahaleb cherry. Peach trees proved an excellent indexing medium for cherry yellows, showing symptoms the year following budding in all cases. On peach, die back, streak, and spot necrosis in the cortex and retarded foliation are characteristic of all strains of the virus. In addition, chlorotic mottling, leaf distortion, shortened internodes, and dwarfing of the tree result from 2 strains. One strain is milder on peach, another produces ring spot on Mahaleb leaves. Peach trees did not react to inoculations from normal cherry trees. Known varieties of peach, such as Hale and South Haven, reacted uniformly to a given strain of cherry yellows, while seedling peaches reacted variably. Eight soil treatments were ineffective in control. Cherry yellows reduced fruit yield by half in observed cases.

The Effect of Cotton-Seed Dusting on Emergence of Seedlings in Soil Infested with Rhizoctonia. RAY, W. WINFIELD. The effect on the emergence of cotton in soil heavily infested with *Rhizoctonia solani*, after treatment of the seed with various kinds of proprietary dusts, was determined in greenhouse experiments. All results obtained were analyzed by the analysis-of-variance-method. Some organic-mercury dusts increase more than do others the emergence of seedlings over the nondusted seed. Copper dusts are somewhat effective, but less so than the best of the mercury compounds. Spergon was the most effective of all those dusts lacking a heavy metal as the active ingredient. It was equal in effectiveness to the best organic mercury dust. Seeds dusted with the various agents tested gave a significantly greater emergence than nondusted seed in nearly every instance, but, so far as the final stand was concerned, dusted seed had little value as a protectant against *Rhizoctonia.*

Control of Diseases of Garden Roses. ROSEN, H. R. During a 4-year period, red cuprous oxide dust mixture (4 per cent cuprous oxide, 2 per cent calcium arsenate, 10 per cent flour, and 84 per cent talc) applied once a week during the growing season, gave excellent control of black spot on susceptible hybrid teas, without injury to foliage under Arkansas conditions. Of numerous fungicides under investigation, it is the only one that gave such results. Breeding for disease resistance has resulted in a number of winter-hardy and mildew-resistant hybrids. Most of these hybrids are climbing types resulting from crosses between *Rosa setigera* derivatives and various bush hybrid teas. *R. setigera* appears to offer an excellent source of resistance to winter injury, powdery mildew, and drought and heat injury but not to black spot. Floral characters of its hybrids are diverse, some desirable, others not.

Crown-rust Infection of Oats as Related to Reduction in Hardiness and Yield. ROSEN, H. R., AND L. M. WEETMAN. Susceptible varieties of oats planted early in September, 1940, became infected with crown rust within 6 weeks, the infections averaging over 40 per cent of leaf areas. The same varieties planted in October showed few or no infections. Along with susceptible varieties, some 5,000 selections of oat hybrids, bred primarily for crown-rust resistance, were also planted early and late. With no opportunity for hardening-off prior to a severe November freeze, all plants of hardy varieties heavily infected with crown rust showed a large amount of frost injury, while plants of the same varieties with little or no crown-rust infection showed a correspondingly lower amount of frost injury. Hardy, crown-rust-resistant strains of both early and late plantings showed a minimum of such injury. The fall epidemic of crown rust on susceptible varieties resulted in a reduction in winter pasture and apparently in grain yields. In the absence of an epidemic in the spring of 1941, susceptible varieties planted late in the fall of 1940, which had escaped the fall epidemic, gave larger grain yields than the early fall plantings. Hardy crown-rust-resistant strains planted early or late in the fall gave exceptionally high yields.

The Influence of Temperature, Moisture, and Soil Reaction on Damping-off of Red Pine by Pythium and Rhizoctonia. ROTH, L. F., AND A. J. RIKER. In Wisconsin forest nurseries *Pythium irregulare* and *Rhizoctonia solani* were the principal causal agents of damping-off. The predominance of one over the other sometimes occurred in different nurseries at the same time, and sometimes in the same nursery at different times. Since the activity of one or the other apparently depended on local conditions, investigations were made of the effects that some important environmental factors had on the development of disease. Greenhouse studies with seed of red pine, planted in Plainfield sand, showed that within limits the maximum damping-off from *Pythium* occurred below 24° C.

and that from *Rhizoctonia* above 24° C. Soil moistures somewhat less than 70 per cent moisture-holding capacity were favorable to *Rhizoctonia* and those more than 70 per cent were favorable to *Pythium*. Saturated air humidity favored *Rhizoctonia*, but did not influence *Pythium*. Within limits soil reactions that were more acid than pH 5.8 were favorable to *Rhizoctonia*, those more alkaline than pH 5.8 were favorable to *Pythium*. The red-pine seed grew well in soil between about 15° and 30° C., with medium moisture, and with a reaction between about pH 4.7 and 6.0. Damping-off was favored, within limits, when emergence and maturity were prolonged by the environment.

Loss of Sporulation in Cercospora. RYKER, T. C. Cultures of certain species of *Cercospora*, regardless of whether they originate from tissues of the host or from single spores, eventually produce feebly sporulating mycelial variants that overgrow the cultures. Very often, in transferring, the original types are lost. The original type, however, can be maintained by transferring from hyphal tips or from conidia. The most common variant is a white mycelial non-conidial form, which usually originates as a patch variant in old cultures. This white variant remains fixed. It has been found common to a number of species of *Cercospora*. From a study of 38 single hyphal-tip isolates of germinating conidia of *C. oryzae*, *C. beticola*, *C. apii*, and *C. nicotiana*, it has been determined that all produce these white mycelial variants, which are remarkably similar in culture. As the cells of the conidia are uninucleate, it is assumed that these variants are mutations. The loss of sporulation in cultures of these species of *Cercospora* is ordinarily due to the development of the non-conidial variants that overgrow the original cultures.

The Importance of Seed Transmission of Early Blight and Fusarium Wilt of Tomato. SAMSON, R. W., T. J. NUGENT, AND L. C. SHENBERGER. The possibility of important transmission of *Alternaria solani* and *Fusarium lycopersici* inside of seed from tomato fruits sufficiently free of decay for juice and purée manufacture was found to be extremely remote, even though the fruits were sorted from crops infected with early blight and fusarium wilt. No seeds internally infected with *A. solani* were found among 15,590 extracted commercially from such tomatoes. Only 2 were found among 5659 plated from seed lots from cull fruits and portions of fruits showing abundant early blight infection. Four of 400 seeds from selected decayed regions of infected fruits yielded pathogenic cultures. No internal infection by *F. lycopersici* was demonstrated in 20,207 seeds from commercial lots saved from seed-production fields containing wilt-infected plants. Colonies of *Fusarium* grew from 4 out of 3690 seeds plated from ripe, edible fruits taken from infected pedicels or stems. No *Fusarium* was secured from 675 seeds removed aseptically from fruits evidencing vascular invasion. The usual methods of surface disinfection with calcium or sodium hypochlorite and mercuric chloride were used for the most part in the plating of seed to determine internal infection.

Comparative Reactions of Single Crosses of Dent Maize to Diplodia zeae. SEMENIUK, G. Forty-nine single crosses of dent maize were tested for comparative reactions to *Diplodia zeae* in 1941 by a greenhouse seedling-infection method and a field stalk-inoculation method. Greenhouse tests were conducted in freshly steamed 2:1 field soil-sand mixture placed in 4-in. unglazed flower pots. *Diplodia zeae* soil-cornmeal inoculum and maize seeds were planted simultaneously. The inoculum was placed as an even layer, 5 g. per pot at the seed level in one group of pots, and 20 g. per pot at 2 cm. below the seed level in another group. The seedlings were examined for infection after 3 weeks. In field tests stalk inoculations were made with an aqueous spore suspension at the fourth internode above the ground level on August 12–13. On September 18–20 the development of *D. zeae* in the pith was measured after splitting the stalks. Significant differences in reaction of the single crosses were obtained in (1) the extent of mesocotyl and primary root necrosis in seedlings when the inoculum was placed at the seed level, (2) the extent of spread and rotting of the pith of stalks after inoculation, and (3) the number of dead stalks resulting from natural causes. No significant interrelationship was found between greenhouse and field data, whereas significant correlations were obtained in the field data.

Diplodia Epidemic in Conifer Seedbeds. SLAGG, C. M., AND ERNEST WRIGHT. Dead and dying seedlings of *Pinus nigra*, *Pseudotsuga taxifolia*, *Pinus ponderosa*, and *Pinus edulis* in seedbeds at a Federal nursery at Manhattan, Kans., were found infected with a fungus identified as *Diplodia pinea* (*Sphaeropsis ellisi*). The same fungus was found on 2- and 3-year-old stock of *Pinus sylvestris*, *P. nigra*, and *P. ponderosa* at the nursery, and on 10- to 50-year-old trees of *P. nigra*, *P. ponderosa*, and *P. sylvestris* on the campus of Kansas State College at Manhattan. In the seedbeds, injury was most severe on *Pinus nigra* and *Pseudotsuga taxifolia*, approximately 50 per cent of the seedlings being diseased or dead. These seedbeds had been sown with dry seed 5 months before the epidemic was observed and the young plants had not yet completed development of woody tissues.

Disease symptoms were reproduced in 5 days on similar seedlings of *P. nigra* from an uninfected nursery inoculated with mycelium of single-spore cultures of the fungus.

Cross Inoculations with Pythium arrhenomanes from Cereals and Grasses in the Northern Great Plains. SPRAGUE, RODERICK, AND R. E. ATKINSON. Forty of 600 isolates of *P. arrhenomanes*, selected to include those from 18 species of cereals and grasses collected at various locations in the Northern Great Plains, were parasitic on all hosts in cross inoculations in the greenhouse. Isolates from durum wheat, emmer, oats, rye, proso, *Agropyron cristatum*, *A. repens*, *Bromus inermis*, and *Elymus junceus* were particularly virulent on all hosts tested. Pre-emergence blight was most severe—often 100 per cent—on *Bouteloua gracilis*, *Oryzopsis hymenoides*, *Panicum miliaceum*, *P. virgatum*, *Phleum pratense*, *Setaria italica*, and *Stipa viridula*. Corn, wheat, sorghum, sudan grass, *A. cristatum*, *Bromus inermis*, and *E. junceus* showed less pre-emergence blight, but were invariably definitely stunted. In the case of *A. cristatum*, *B. inermis*, and *E. junceus*, injury was much greater when old, small, or moldy seed was used, seed strain causing more variation in root rot than the source of the fungus. (Cooperative investigations of the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry-Land Agriculture, Bureau of Plant Industry, and the Nursery Division, Soil Conservation Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.)

Recent Changes in Prevalence of Physiologic Races of Puccinia graminis tritici in the United States. STAKMAN, E. C., AND W. Q. LOEGERING. Race 56 of *Puccinia graminis tritici* increased in prevalence in the United States each year from 1930 to 1938, inclusive, and was the most prevalent race from 1934 to 1940, but it began to decrease in 1939 and in 1941 was surpassed by race 17, which had shown a tendency to increase gradually from 1930 to 1939 and then increased rapidly from 10 per cent of all isolates in 1939 to 34 per cent in 1940 and 52 per cent in 1941. If these trends continue, Thatcher wheat should retain its resistance in the spring wheat region, as it is highly resistant to race 56 and immune from race 17, which together comprised 84 per cent of all racial isolates in 1941; and, taking all races into consideration, it either is immune from or resistant to 94 per cent of those isolated. On the other hand, the commonly grown durums are resistant to only 37 per cent of the isolates obtained in 1941, as contrasted with about 70 per cent in 1938 before the decided increase of race 17, to which they are susceptible, and the decrease of race 56, to which they are resistant. It is not improbable, therefore, that stem rust may again become important in the durum wheat region. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Inactivating in Vivo the Virus of X-disease of Peach by Chemotherapy. STODDARD, E. M. Since 1935, 45 organic and inorganic chemicals have been used to treat living peach tissue infected with the virus of X-disease. These chemicals were applied chiefly by soaking 10 randomized diseased buds in water solutions of the chemicals. Nearly all the buds grew when budded into healthy seedlings. The most complete experiment was made in 1941. Eight of the 10 diseased buds, soaked in quinhydrone, failed to produce the disease as compared to 1 in the checks and none in some of the treatments. 8-hydroxyquinoline sulphate, hydroquinone, p-nitrophenol, Calcium 8-hydroxyquinolate, urea, sodium thiosulphate and some of their derivatives inactivated the virus in lesser degree, under like conditions, at the concentrations used. In a preliminary test, in 1940, buds soaked in urea, calcium 8-hydroxyquinolate, magnesium 8-hydroxyquinolate, o-nitrophenol, and sodium thiosulphate have failed to produce the disease to date. In these experiments most of the buds failing to produce the disease grew and developed normal healthy shoots. These data show that the virus of X-disease of peach can be inactivated *in vivo*, by chemicals, and that diseased tissues will recover and grow normally. They offer the hope that other viruses will respond similarly.

The Thread-blight Fungus, Corticium stevensii. TIMS, E. C. The thread-blight fungus, *Corticium stevensii*, is found on a number of host plants in Louisiana. Cultures obtained from sclerotia from several of these hosts have been quite similar in culture and in pathogenicity on fig leaves and twigs. Single-basidiospore cultures obtained from fig leaves also have been uniform in cultural characters, and in all cases similar to the cultures obtained from sclerotia or from spore masses. The monosporous cultures produced basidial mats with typical basidiospores when inoculated individually on fig leaves. Many of these cultures also produced typical hyphal threads and sclerotia on fig twigs when the leaves were inoculated. The constant similarity in all cultures also produced typical hyphal threads and sclerotia on fig twigs when the leaves were inoculated. The constant similarity in all cultures of this fungus, whether obtained from sclerotia, spore masses, or single basidiospores, and the uniform way in which the monosporous cultures produced

normal fruiting structures on fig leaves indicates that *C. stevensii* is, apparently, a homothallic form.

Studies in the Classification of the Bacterial Plant Pathogens. WALDEE, E. L. Comparative studies of phytopathogenic bacteria having peritrichous flagella revealed the existence of at least 3 easily recognizable generic groups. The genus *Erwinia* in the restricted sense suggested in a previous abstract does not fit into any of the existing families of bacteria. It is proposed, therefore, that a new family in the order Eubacteriales be recognized with *Erwinia* designated as the type genus. The soft-rot bacteria were found to be more closely related to the coliform bacteria than to the members of the genus *Erwinia*. Because they constitute a distinct taxonomic unit it is proposed that the soft-rot bacteria be incorporated into a separate genus in the family containing the coliform bacteria. The yellow organisms, including *B. lathyri*, *B. ananas* and *Bact. stewartii*, are not yet well understood and occupy a doubtful position.

The Classification of the Cornstalk-rot Pathogen. WALDEE, E. L. Eleven isolates of the cornstalk-rot pathogen were studied comparatively together with more than 40 isolates of soft-rot bacteria and representative cultures of 4 species of coliform bacteria. The results of this study show that the cornstalk-rot pathogen is not a soft-rot organism, but that it is a member of the coliform group of bacteria, usually nonmotile, gram-negative, and rod-shape. Glucose, lactose, and cellobiose are fermented with production of acid and much gas (not less than 10 per cent and usually more than 50 per cent in Durham tubes). Nitrates are reduced to nitrites. The organism does not produce indol, is methyl red-negative, Voges-Proskauer positive, and grows well in Koser's citrate medium. No protopectinase is secreted. These and other reactions indicate that the organism is a member of Aerobacter section of the coliform bacteria.

Physiologic Races of Plasmodiophora brassicae. WALKER, J. C. Several varieties of turnip and rutabaga tested with various collections of *P. brassicae* from widely separated localities in the United States were found to remain completely free from development of clubs when grown in heavily infested soil. One of these, Purple Top Milan turnip, grown on naturally infested soil in 2 locations in England developed about 20 per cent diseased plants in each location. An English variety, White Stone, which showed 87 per cent diseased plants in an English test, failed to develop any clubbed roots with a representative American isolate. This is submitted as proof that definite physiologic races of *P. brassicae* exist. The tests in England were carried out by F. T. Bennett in the North of England and by N. C. Preston in Shropshire. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, and University of Wisconsin.)

Systemic Invasion of Cabbage by Plasmodiophora brassicae. WALKER, J. C. Under greenhouse conditions when cabbage seedlings are grown in soil infested with *P. brassicae* the pathogen, after infecting the root, may migrate through the cambium into the stem. There is relatively little cambial proliferation in the internodal regions above the third or fourth leaf. Dormant buds at the leaf scars, however, are stimulated to grow, and become invaded by the pathogen. They become malformed due to extreme hyperplasia. The organism may reach the growing point in young plants and cause extreme distortion of stem and leaves. When plants are inoculated at aboveground leaf nodes, the pathogen may migrate down the stem, leaving no evidence of proliferation in its path until the hypocotyl is reached, where a typical club is formed. There is evidence that the reaction of the host is influenced by the nutrient supplied to it. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, and University of Wisconsin.)

Virus Strains in Relation to Acquired Immunity from Curly Top in Tomato. WALLACE, J. M. Plants of cultivated varieties of tomato (*Lycopersicum esculentum*) very rarely recover and acquire an active immunity from curly top. However, they can be passively immunized by grafting with tobacco (*Nicotiana tabacum*) plants that have acquired an immunity. The clonal progeny of passively immunized tomato plants, when grown in the field under severe curly-top exposure, showed a high degree of protection, but occasional plants within an immunized clone developed severe curly-top exposure, showed a high degree of protection, but occasional plants within an immunized clone developed severe curly top. This led to a study of strains of the virus, which showed that tomato plants immunized against single virus strains were either unaffected, mildly affected, or quite severely affected, depending upon the strain of virus used for reinoculation. Healthy, non-immunized plants were severely affected by all of the virus strains used for immunization and reinoculation. This role of virus strains in the field of acquired immunity in plants parallels certain reactions in the animal virus field, where strains of a given virus differ antigenically, the immune-sera of the different strains not providing

cross immunity. It is believed that these data furnish additional evidence that acquired immunity from curly top results from the production of some type of protective substances.

Genetic Studies in Oats of Resistance to Two Physiologic Races of Crown Rust. WEETMAN, L. M. Inheritance studies have been made of the types of resistance to crown rust found in 3 varieties of oats used as resistant parents in breeding work. These varieties are Bond (resistant to race 1, susceptible to race 45), Victoria (resistant to both races 1 and 45), and Mutica Ukraina (resistant to race 45 and to a portion of race 1). F_2 and F_3 data indicate that the Bond type of resistance to race 1 is conditioned by two dominant complementary genes. Victoria resistance to both races 1 and 45 appears to be largely determined by a single factor which is distinct from the Bond genes. F_2 field data indicate that the Mutica resistance to the major portion of race 1 is due to two dominant complementary genes as in Bond. These genes are probably allelomorphic to the Bond genes for resistance.

Further Studies on Pythium Injury of Oats. WELCH, AARON. Under Iowa conditions *Pythium debaryanum* and related species of *Pythium* have proved to be serious parasites on the roots of oat plants. From 1937 to 1941 these organisms were prevalent throughout the State and were isolated each year from oats growing in widely separated localities. Under controlled greenhouse conditions 218 varieties of oats (including winter, spring, and wild types) were grown in steamed soil artificially infested with *P. debaryanum*. Marked resistance was not observed in any one variety, but some varieties were more resistant than others. The effects of phosphorous, potash, nitrogen, manure, and a complete fertilizer on the ability of *P. debaryanum* to parasitize oat roots failed to indicate that any of the treatments were beneficial in reducing or controlling infection. Oats grown in plots treated with chlorpicrin, however, yielded 100 per cent more than the same varieties grown in non-treated fertilized plots.

Some Tentative Conclusions Resulting from Plot Analyses of Phomopsis-blighted Juniper Seedlings in Great Plains Nurseries during 1941. WRIGHT, ERNEST, AND C. M. SLAGG. Because of need for information on control of Phomopsis blight of junipers in broadcast sowings, the following tentative conclusions may be of interest. Contrary to current opinion, 1-year-old eastern red cedar (*Juniperus virginiana*) seedlings originating from Kansas-collected seed proved no more resistant to infection by *Phomopsis juniperovora* than those grown from Nebraska seed. Likewise, western red cedar (*J. scopulorum*), previously not sprayed because it was considered highly resistant to the disease, was so severely infected that spraying will be necessary in the future. Infection of juniper seedlings usually started at the margin of the seedbeds, eventually worked inward, and appeared to be directly associated with water splashing. Seedlings watered by overhead irrigation were, therefore, more severely blighted than those watered by ditch irrigation. Dense stands, presumably because they reduce water splashing, tended to retard rather than to increase the percentage of infection. Roguing of diseased plants was not significantly beneficial except in plots where infection was relatively light. To be most effective, the seedlings should be rogued as soon as they show the first symptoms of infection before sporulation begins. Of 7 sprays used, commercial Bordeaux (5–5–50) gave the most promise of controlling the disease.

Stimulatory and Toxic Effects of Copper Sprays on Powdery Mildews. YARWOOD, C. E. The dried deposit of 0.1 per cent Bordeaux $\pm$ 0.1 per cent cottonseed oil on glass slides was toxic to conidia of *Erysiphe polygoni* from red clover and bean in water, approximately neutral on dry slides at 100 per cent R.H., and stimulatory at 90 per cent R.H. Copper sulphate of Bordeaux added to sucrose agar stimulated the germination of bean powdery-mildew conidia, but inhibited the growth of contaminating fungi. On inoculation, beans sprayed with 0.1 per cent Bordeaux frequently showed more mildew development on the upper surface of primary leaves than did unsprayed plants. This stimulatory effect of Bordeaux was greatest when light was inhibitory to mildew development, though the nature of the inhibition by light is not understood. Many fungicides, including Bordeaux and lime sulphur, were more effective as eradicant sprays for powdery mildew on beans, cucumbers, and cantaloupes, and for rust on bean and sunflower on plants held in moist chambers for several hours after spraying than on plants dried soon after spraying. Most fungicides tested were more effective as eradicant than as protective sprays for powdery mildews.

Fungicidal Value of Bordeaux Mixtures Prepared from Diluted and Concentrated Stock Solutions. YARWOOD, C. E. Concentrated bluestone Bordeaux prepared by adding 13 per cent $Ca(OH)_2$ to 10 per cent $CuSO_4 \cdot 5H_2O$ and then adding the required amount of water, was compared with diluted bluestone Bordeaux prepared by adding 13 per cent

$Ca(OH)_2$ to $CuSO_4 \cdot 5H_2O$, which had first been diluted to spray strength, as protective sprays for onion downy mildew, hop downy mildew, cucumber downy mildew, cucumber powdery mildew, bean powdery mildew, and bean rust, and as eradicant fungicides for bean powdery mildew and cucumber powdery mildew in greenhouse tests under conditions of heavy artificial inoculation. In 66 paired comparisons there was little difference in the fungicidal value of the diluted bluestone Bordeaux and concentrated bluestone Bordeaux. Mixtures containing 0.03 to 0.3 per cent bluestone gave marked control of all diseases, except as protective sprays for bean mildew. Only in the case of bean rust was the diluted bluestone Bordeaux markedly superior to the concentrated bluestone Bordeaux. The addition of certain spray supplements increased the protective value of most sprays but did not affect the relative merit of diluted bluestone and concentrated bluestone Bordeaux.

Toxin Formation and Chemotherapy in Relation to Dutch Elm Disease. ZENTMYER, GEORGE A. *Ceratostomella ulmi* was found to produce in culture a toxin that wilts plant cuttings and induces symptoms similar to those of the Dutch elm disease when injected into elms. This suggests that the fungus produces in the tree a similar toxin, responsible for disease symptoms. In chemical treatment of 850 small (3–6-ft.-tall) American elms, 5 of 25 organic chemicals gave promising results in preventing or retarding progress of disease. Injecting hydroquinone, benzoic acid, or p-nitrophenol, 1 week before inoculation with *C. ulmi*, greatly reduced the percentage of trees showing disease symptoms as compared with water-injected controls. When 50 trees were injected with 8-hydroxyquinoline sulphate and simultaneously inoculated, the appearance of symptoms was significantly retarded for 2½ weeks, as compared with 50 noninjected controls. When inoculated trees were injected with benzoic acid, hydroquinone, or 8-hydroxyquinoline benzoate at the onset of wilting, the further advance of wilting was markedly retarded as compared with water-injected controls. Retardation of disease by injection of these chemicals offers hope that the Dutch elm disease and similar vascular diseases may ultimately be controlled by chemotherapy. Results indicate that once the disease becomes well established the possibilities of checking its advance by chemotherapy are considerably diminished.

ABSTRACTS PERTAINING TO DEMONSTRATIONS

Western Red Rot in Ponderosa Pine in Arizona and New Mexico. ANDREWS, STUART R., AND LAKE S. GILL. Western red rot, caused by *Polyporus ellisianus*, is responsible for a major proportion of the 25 per cent defect frequently found in mature and overmature ponderosa pine stands in the Southwest. Studies during 1938 and 1939 to determine its importance in immature stands indicated that, since western red rot enters trees almost exclusively through dead branches, initial infection is but slightly contingent on age and suppression, but is highly dependent on the branch habit of trees. In the 41–100-year age class, of the trees with only dead branches less than 1.1 inches in basal diameter, 8.0 per cent had infected branches, as compared with 33.8 per cent of those having one or more branches larger than that size. Although infection also was correlated with tree age, size, and stand density, this may be attributed to the high correlation between these factors and branch habit. Age alone was found highly correlated with the progress of decay in the heartwood of the trunk. Studies made during the second cutting of a tract in northern Arizona indicated that the proportions of gross volume lost because of western red rot amounted to 0.8 per cent in trees 121–150 years old, 4.6 per cent in those 151–180 years old, and 10.3 per cent in those 241–270 years old. It would appear that while western-red-rot losses may be somewhat less severe in second-growth than in old-growth stands, they can be further reduced if stands are kept dense enough to inhibit large-branch formation, pruned at an early age, and harvested before they are overmature.

Cross-Inoculations with Fusarium-wilt Organisms. ARMSTRONG, G. M., B. S. HAWKINS, AND C. C. BENNETT. Fusarium-wilt organisms were obtained from the following plants: cotton, okra, watermelon, sweet potato, tobacco (3 sources), tomato, mimosa (*Albizzia julibrissin*) and coffee weed (*Cassia tora*). Inoculations of all hosts, except mimosa, were made either in soil or water culture with at least 3 of the organisms. Records were made of external symptoms of wilt, internal darkening, and recovery of the fungus by plating. The isolates from cotton, okra, coffee weed, and tobacco in South Carolina and Kentucky are apparently the same. All cause wilting in the first 3 hosts and in a susceptible Burley tobacco but not in a resistant Burley or Gold Dollar (flue-cured variety). A Maryland tobacco isolate caused wilting of all the above tobacco varieties as well as Maryland Mammoth but did not affect cotton or okra. The cotton fungus, however, caused wilting of Maryland Mammoth tobacco. The sweet-potato fungus did not cause wilting of cotton, nor did the cotton fungus cause wilting of sweet potato. The sweet-potato fungus, however, caused wilting of a susceptible Burley tobacco. The

tomato fungus failed to cause wilting in cotton, okra, and watermelon and the watermelon fungus failed to cause wilting in sweet potato and tomato.

A Method of Inoculation for Barley Stripe. ARNY, D. C., AND H. L. SHANDS. The stripe reaction of barley varieties and selections may be had from the following test: Sterile water is introduced into a 7- to 12-day-old test-tube slant culture of *Helminthosporium gramineum* and the mycelium scraped off in fine aggregations. Two cc. of this suspension are then added to 125 ml. Ehrlenmeyer flasks previously prepared by adding 15 g. wheat and 15 cc. water, and autoclaving 45 minutes. When fungous growth on the wheat is 5 days old, 25 to 100 kernels of each selection being tested are treated with alcohol to remove surface contaminants, rinsed in water, and placed in one of the flasks. Kernels in contact with inoculum are incubated 4 days at room temperature (20 to 24° C.). Lower temperatures produce more infection if time be adjusted. Flasks are shaken daily to prevent clumping caused by mycelial and root growth. Larger flasks may be used to accommodate more kernels. The entire contents of the flasks are planted in soil. A susceptible variety, such as Oderbrucker, usually has 80 per cent or more infection. Ordinarily about 50 per cent of the kernels planted in the field produce plants, while over 80 per cent may be expected in the greenhouse.

Bacterial Necrosis of the Giant Cactus. BROWN, J. G., LAKE S. GILL, PAUL C. LIGHTLE, AND DON M. HEEP. Work on bacterial necrosis of the giant cactus (*Carnegiea gigantea*), reported by the senior author and co-workers at the last Philadelphia Meeting, A. P. S., has been cooperatively organized by the Arizona Agricultural Experiment Station and the Bureau of Plant Industry, U. S. Department of Agriculture. Survey of large plots in the Saguaro National Monument have been completed in which the incidence of necrosis has been determined and mapped. Activities in attempted eradication or control of the disease include sanitary measures and surgery, in cases of early stages of infection. Necrosis in the Monument and in other giant-cactus forests in Arizona is now very active. Species of *Opuntia* thus far inoculated with the proved cause of necrosis in *Carnegiea* have shown no symptoms of the disease.

Response of Diseased Maple Trees to Chemotherapy and Fertilization. CAROSELLI, N. E., AND F. L. HOWARD. Average twig elongation has shown highly significant differences when maple trees afflicted with bleeding canker were given the following treatments: (1) Injected with antidotal chemicals and subsequently fed by the soil crow-bar method; (2) injected and not fed; (3) fed and not injected, and (4) left untreated. Five mature trees were used for each treatment and the twig growth measured for each of the 6 years 1936 to 1941, inclusive. Ten samples of twigs taken at random from each of the top, middle, and lower sections of each tree were measured. No significant difference was found to exist within treatments. Twig elongation (1) was about uniform for the healthy trees; (2) gradually decreased about one-half during the 6 years for the untreated diseased trees; (3) decreased to the minimum for diseased trees fed but not injected; (4) increased somewhat during the current year and sharply the following year for diseased trees that were injected; and (5) was greater during the year injected and increased most markedly in the next year for both injected and fed. Improvements in injection technique will be demonstrated.

Masking of Wheat Leaf-rust Infections by High Temperatures. CHESTER, K. STARR. Wheat seedlings inoculated with leaf rust (*Puccinia triticina*) and then incubated at temperatures above or below the optimum for rust production, frequently fail to manifest infection, leading to erroneous conclusions regarding host susceptibility or race identity. In an attempt to understand this temperature-conditioned inhibition of rust reaction, seedlings of the 8 wheat varieties used in leaf-rust race differentiation were inoculated with races 5, 19, 20, and 34 and incubated at various temperatures between 39° and 77° F. Highly susceptible varieties, when incubated at 55° F., showed in numerous cases no infection if incubated for 24 hours at higher temperatures and then exposed to optimum temperatures for long periods, indicating that at the higher temperatures no infection occurred. When similarly inoculated plants were exposed to 55° F. for the first 24 hours and then transferred to higher temperatures for 10 days or longer, no infection was apparent, but rust pustules promptly appeared whenever such plants were returned to 55° F., indicating that infection occurred during the first 24 hours at 55° F. but became latent at higher temperatures.

Behavior of Dwarf Mistletoes of Western Conifers in Relation to Control. GILL, LAKE S., AND STUART R. ANDREWS. The dwarf mistletoes (*Arceuthobium* spp.) are so abundant and harmful in parts of the West that there is much practical interest in the effect of old infected trees left standing after logging on the infection of the young stand.

In ponderosa pine, in northern Arizona, where a heavily infected stand had been logged 30 years earlier, data were obtained in 1939, on an area of 60 acres, on the behavior of *A. vaginata* forma *cryptopodum* in the understory of seedlings and saplings. The majority of these had not become established until some time after the original cutting; infection in them was unusually low and for the most part restricted to stands within a radius of 50 feet of infective overstory trees. The parasite had had no significant influence on the height of seedlings, largely because almost all infections were of recent origin, no infection having occurred prior to 1926. Nevertheless, since 70 per cent of the infections occurred on the main stems of seedlings, mistletoe will eventually cause deformity or serious reduction in growth, if not death. In addition, about one-half of those in branches were so close to the main stems as to indicate extension of the endophytic system into the trunks.

Symptom Expression in Psorosis of Citrus as Related to Kind of Inoculum. FAWCETT, H. S., AND L. C. COCHRAN. Sweet-orange trees developed from buds of trees infected with the virus of psorosis A (*Citrivir psorosis* var. *vulgare*) require an incubation period of 5 or more years before bark lesions appear. In some trees this time may be 20 years; the average is between 12 and 16 years. Older trees inoculated with buds taken from twigs not showing bark lesions on psorosis-afflicted trees also require 5 or more years for bark symptoms to appear. If, however, patches of live bark, cut with a cork borer, from the center of scaling lesions, on a Psorosis A-affected tree are transplanted to trunks of sweet-orange seedlings, bark scaling may begin in less than 5 months. Trees inoculated similarly from normal bark 3 to 6 inches away from the lesions have shown no bark lesions after 3 years. Some indication was obtained that patches taken from the middle showed a shorter incubation period than those from the advancing edge of the lesion. Inoculation with the virus of Psorosis B (*Citrivir psorosis* var. *anulatum*) gave corresponding but shorter incubation periods. This method not only opens a new approach to the character of the psorosis virus, but suggests a method of greatly shortening the incubation period.

Fumigation of Potting Soils. GODFREY, G. H. Soils completely free from nematodes and from damping-off and root-rot fungi, and practically free from weeds, can be kept available for use by the simple and relatively inexpensive means of fumigation. Chloropicrin or methyl bromide at 4 ml. per cu. ft. have been found highly effective for killing soil fungi, as well as nematodes (*Heterodera mariona* and *Pratylenchus pratensis*). Carbon bisulphide and ethylene dichloride at 10 ml. per cu. ft. have killed nematode infestations satisfactorily, but are not so reliable against fungi. Recent tests with methyl bromide have indicated that, while it is difficult to handle because of its low boiling point, it is highly promising for soil fumigation because of its apparent ability to penetrate undecayed nematode galls. Galvanized cans or gas-tight boxes are suitable for soil containers for fumigation. In all cases means must be provided for sealing a gas-tight cover. The soil should be loose and free from excessive wetness, and fumigation done while temperature is high. Applicators are available at much lower cost than heat-sterilizing equipment.

The Relation of Certain Weevils to Root Rot and Basal Stem Rot of Cereals and Grasses. HANSON, E. W., AND H. E. MILLIRON. Certain weevils infesting Gramineae are important in the development of root rot and basal stem rot of cereals and grasses in the Northern Great Plains. Several species of these weevils are found in this area, and it is known that some of them attack many hosts. The widely distributed *Calendra parvula* is an important species recorded in our investigations as attacking wheat, timothy, bluegrass and crested wheatgrass. Most of the insect injury is confined to the lower internodes of the culms. Very severe rotting is consistently associated with such infestation. The work of this weevil provides avenues of entrance for fungi and bacteria and promotes the development of rots in the basal parts of the plants. The infested internodes are filled with frass, resulting from larval feeding, which is an excellent medium for the rapid increase of microorganisms. The dissemination of these rot-inducing organisms appears to be facilitated by such feeding and the movement of the larvae inside the plants. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Plant Pathology and Botany, and Entomology and Economic Zoology of the Minnesota Agricultural Experiment Station.)

Antidoting the Toxins of Plant Diseases. HORSFALL, JAMES G., AND GEORGE A. ZENTMYER. It is known that many of the pathogenic effects in plant diseases are produced by toxins, and that even the parasitism of many organisms may be possible only because excreted substances precede the invader into the host, killing the tissue and preparing the way for further advance. Research in 1940 and 1941 indicated that toxins of

Dutch elm disease and Verticillosis of eggplant and maple may be weakened, possibly eliminated, by internal chemotherapy with the following compounds and their derivatives containing basic nitrogen: 8-hydroxyquinoline sulphate, urea, dihydrochloride of diaminobenzene, malachite green, and probably ammonia. Since the effects of several root diseases are known to be reduced by ammonia, apparently through some non-fungicidal mechanism, these results suggest that such root diseases are toxin-induced and that the basic nitrogen in ammonia antidotes the toxin. These results also suggest an additional approach to chemical control of plant disease. By and large, chemicals are applied externally to the host, *i.e.*, aimed at killing potential parasites. Chemicals are applied internally here, more with the aim of interfering with the mechanism of pathogenesis than of killing the parasite.

Life Activities and Control of Phytophthora citrophthora. KLOTZ, L. J., AND H. S. FAWCETT. Hyphae grown on alfalfa stems produce abundant functional sporangia when placed in well-aerated water. Sudden lowering of temperature causes mature sporangia to discharge zoospores. A swimming zoospore takes a spiral course and revolves continuously on its long axis. The cilium, extended forward, propels by means of a corkscrew motion; the other trails, rudderlike. Either can assume the function of the other, making it unnecessary to about face to reverse direction. Germination produces 1 to 5 germ tubes. Diplanetism is frequently exhibited. Under favorable temperature and moisture any organ (bark, fibrous roots, leaves, flowers, and fruit) can be infected at any point, no wound being necessary. Hyphae invade inter- and intracellularly. Control measures are based on zoospore sensitivity to heat and euprion. Grove protection is afforded by copper sprays, good drainage, aeration, and sunlight. Sunlight raises exposed bark temperature 15° to 25° F. above air temperature and, in summer, kills invading fungus. Rind of lemons can also be heated to such lethal temperatures by immersion in water at 118° to 120° F. In storage, contact infection by aerial hyphae can be suppressed by prompt removal of rots, lowering humidity to 75 to 80 per cent, and maintaining a concentration of 1 to 2 mg. of NCl_3 gas per cu. ft. for 4 hours at weekly intervals.

Production of Disease-resistant Sorghums. MELCHERS, L. E., F. A. WAGNER, AND A. E. LOWE. The Southern Great Plains area produces several million acres of milo and milo hybrids for grain. They are susceptible to the milo disease. The Kansas Agricultural Experiment Station, in cooperation with the Division of Cereal Crops and Diseases, U. S. D. A., has studied the cause and control of this malady. The exhibit illustrates the various steps followed in selecting resistant plants in the field and greenhouse, how these selections are further tested to eliminate accidental "escapes," segregates, or varietal mixtures, and how disease reaction and agronomic character may be determined in infested soils. The use of greenhouse methods demonstrates how selection for resistance may be accomplished and how these methods are used in testing certified seed of resistant strains of milo. Photographs show how county agents and growers may have soil from questionable fields tested for the presence of the disease. Living plant material of resistant selections produced at the Kansas Agricultural Experiment Station will be shown.

Seed Treatment of Good Seed Corn. REDDY, C. S., AND W. N. RICE. Seed treatment of good seed corn is beneficial when adverse conditions prevail at planting time. Treatment is of little or no value when the seed is planted in moist or dry soil that remains warm for several days. When the seed is planted in somewhat dry soil that remains cold for several days (in demonstration 7 days at 10° C.), seed treatment is of value in obtaining better field stands. The value of seed treatment increases under cold soil conditions with increases in soil moisture. The limit of value is reached when stands from treated seed are entirely satisfactory and stands from non-treated seed are so poor as to necessitate replanting. The demonstration shows the effect of seed treatment using a commercial organic mercury dust, New Improved Semesan Jr., and an experimental organic dust, Spergonex. Seed lots vary in degree of response to temperature and moisture. This degree of response is the basis for the seed-laboratory "cold-test" in which a special tensiometer is used to insure the same moisture factor for each test.

Persistence of Cotton-root-rot Sclerotia Following Certain Cropping Practices. ROGERS, C. H., AND HERBERT RICH. Cotton-root-rot (*Phymatotrichum omnivorum*) sclerotia have been found at depths to 8 ft. in the Texas blackland soils and appear to be one of the most important factors in survival of the fungus. Most of these sclerotia occur in the upper 3 ft., approximately half the total being found at depths of 12 to 24 in. Large quantities of viable sclerotia were found in soil after 12 years of continuous cropping with cereals, which are non-susceptible, and after the same period of clean fallow. Sclerotia may thus retain their viability and infect susceptible plants after lying dormant in the soil for long periods of years. Sclerotial populations varied from a few thousand to 10,000,000 or more per acre foot of soil.

A Comparative Study of Four Species of Rhizoctonia. RYKER, T. C., AND BEATRICE EXNER. Four rhizoctonia diseases in the South can be distinguished on the basis of symptoms produced on various hosts and the appearance of the isolates on culture media. They are the banded sclerotial disease of Bermuda grass, rice, and sugar cane caused by *Hypochnus sasakii;* web blight of beans and figs and other plants caused by *R. microsclerotia;* an undescribed blight of figs caused by a *Corticium* sp., and the various root and stem rots caused by *R. solani.* Studies of the Corticium stages of the 4 have shown no distinct differences either in size and shape of basidiospores or in appearance of basidial mats. Tissue isolates of diseased material have shown very little variation and it has been possible to classify such isolates satisfactorily in the 4 groups. On the other hand, while some cultures from single basidiospores of *H. sasakii, R. microsclerotia,* and the *Corticium* from fig have been similar to the tissue cultures, most of them have been so different that they could not be placed in any one of the 4 groups. Single basidiospore isolates of *R. solani,* however, while showing some variation, could all be classified as *R. solani.* The results suggest the possibility that the 3 former fungi are hybrid forms and that the variation is due to segregation.

Pythium Injury to Flax. SCHLICK, R. W. Flax develops poorly following sugar beets. Stands are poor and the developing plants lack the vigor and color of healthy flax plants. In searching for the cause of this condition a species of *Pythium* (*P. debaryanum* group) was obtained in a high percentage of the isolations made from seedlings. In many cases, before the seedlings had emerged, the radicle was discolored and the cotyledons rotted within the seed coat. In less severe cases only the tip of the radicle showed a reddish-tan discoloration, and these seedlings emerged. In the former case it appeared that this species of *Pythium* can cause a direct general necrosis. Isolates from more mature plants in the spring and early summer were predominately *Pythium* sp., sometimes associated with *Rhizoctonia* sp., *Fusarium* sp., *Helminthosporium* sp., etc. The lateral and tap roots showed a light reddish-tan to brick-red discoloration. A few small lateral lesions on the tap root showed similar discolorations. These attacks were restricted to the cortical tissue.

Placement of Diplodia zeae Inoculum in the Soil in Relation to Infection of Maize Seedlings in Greenhouse Pot Experiments. SEMENIUK, G. *Diplodia zeae* soil-cornmeal inoculum was placed as a uniform layer below, at and above the seed level. Inoculum in 5–20 g. amounts and seeds were planted at the same time in potted, freshly steamed sand and soil-sand mixture. Seedlings were examined after 3 weeks at 68–75° F. Severe mesocotyl and primary root necrosis initiated at the point of their juncture was obtained from inoculum placed at or above the seed level. Only few small primary root lesions were obtained from inoculum placed 2 or 4 cm. below the seed. Mean air temperatures of approximately 65, 75, and 85° F. yielded similar infections from inoculum at and below the seed. Sand yielded fewer root lesions from inoculum placed below the seed than did a soil-sand mixture. Soaking seed 10 minutes in a 1: 10,000 ethyl mercury phosphate solution markedly reduced infection from inoculum placed at and above the seed, but did not influence root infections from inoculum below. Isolated necrotic lesions on the mesocotyl were rarely observed. The washing down of spores from the inoculum above the seed was suggested. *Diplodia zeae* was unable to grow sufficiently in steamed soil-sand pot experiments from below the seed to establish infection at the seed level.

Acquired Immunity from Curly Top in Tobacco and Tomato. WALLACE, J. M. A summary of the results of the study of acquired immunity from curly-top virosis is presented and illustrated. Special attention is given to the phases of this investigation in which the plant-virus relations are similar to immunological phenomena in the field of animal viruses.

Wilt-resistant Tomatoes with New Genetic Characters. YOUNG, P. A. Cuttings of *Lycopersicon chilense* × *L. esculentum,* received from F. O. Holmes, bore flowers but no fruit. Pollen from them was back-crossed onto *L. esculentum.* Progeny plants showed intermediate inheritance of finely dissected leaflets; plants with prominently dissected leaflets were sterile. Fruits on the other plants were 1 to 2 cm. in diameter, and yellow or orange; few plants had red fruits, which indicated unusual inheritance of fruit color. Striped fruits appeared on one plant of the variety Michigan State. Stripes and corky pits appeared in the fruit peel of most of the progeny plants of the 2nd and 3rd generations. In a field of Rutgers tomatoes, one plant bore lobed, nearly hollow fruits, 7 cm. in diameter. This fruit character bred true through 3 generations. Apparently resulting from X-ray-induced mutations, one tomato selection had yellowish bordered leaflets. Another selection had white flowers. The white-flower character was transmitted to hybrids nearly immune from Fusarium wilt. One plant of the variety Prairiana bore sticky fruits associated with light-green leaflets and hairy stems. These characters bred true through 3 generations.

THE EFFECT OF CERTAIN CHEMICALS, SOME OF WHICH PRODUCE CHROMOSOME DOUBLING, ON PLANT TUMORS

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INTRODUCTION

Since 1937, colchicine has been used extensively by geneticists to attempt the improvement of such flowers, vegetables, and fruits as will respond to its stimulation by the production of new and desirable qualities. It is extracted largely from the plant *Colchicum autumnale* L., the autumn crocus, abundant in Asia Minor. Early Greek physicians used it as a remedy for gout; even today it is used to a limited extent in the treatment of arthritis. It was a physician treating a tumorous patient for gout who noticed and recorded the fact that colchicine delayed the advance of the tumor. Discovery of this record years afterwards induced some animal research workers to experiment with the alkaloid; they were followed by students of animal cancer, geneticists, and plant pathologists.

Colchicine, in very weak solution, is used by geneticists in the attempt to produce new varieties of plants. The alkaloid induces a doubling of the number of chromosomes in the nucleus. A short exposure to a weak solution limits the amount of doubling that takes place. A strong solution or a long exposure to a weak one would defeat the result hoped for. With a short exposure to a weak solution, cell division continues in the regular manner; and, after division, the new-formed cells contain the increased number of chromosomes. The changed nucleus with its added number of chromosomes has its effect on the plant, and some new and distinct type of variation may be the result. One of the methods for producing new varieties of plants is to dip young growing stems in a 0.2 per cent solution of the alkaloid.

In experimenting on the effect of colchicine on bacterial plant tumors, it was found that it could not only inhibit their growth but also kill them. The simplest and most direct way to introduce the alkaloid was to brush a 2 per cent aqueous solution on the surface of the tumor with a camel-hair brush. When temperature and moisture conditions were especially favorable for penetration, a 1 per cent, or even 0.5 per cent, solution would bring about the desired result. A 3 or 4 per cent solution proved no more effective than the 2 per cent solution, nor did death follow its application any more readily. Brushing the tumors with colchicine solutions of these concentrations had no apparent effect on the other parts of the plants; they grew, blossomed, and produced seed quite normally.

Having studied the relation of colchicine to polyploidy in various plants, my colleague, Haig Dermen, became interested and made a study of the nuclei of cells of colchicine-treated plant tumors before death took place. He found that, at mitosis, the chromosomes continued to divide repeatedly.

There was no indication of spindle formation or migration of chromosomes to the poles or of cross walls forming to produce daughter cells. The separate chromosome divisions massed together in one huge nucleus with irregular lobes (2, 3, 4). He estimated that the normal number of chromosomes—48 for the French marigold, *Tagetes patula* L.—might divide until there could be as many as 24,500 chromosomes holding together in one irregular nucleus, the cell increasing in size correspondingly. As this was an abnormal condition the plant could not overcome, death of the tumor cells occurred, and, eventually, death of the entire tumor followed.

The death of bacterial plant tumors subsequent to brushing them with a 2 per cent solution of colchicine was reported in 1939 (1, 2). With plant tumors as with healthy plant tissue, the cells must be young and actively dividing if the number of chromosomes is to be increased by the application of colchicine. It is apparently the stimulation to excessive doubling without cell division that destroys the balanced metabolism of the cells. This condition is attributable to the many chromosomes massed together in the huge lobed nucleus; and it follows that the tumor cells increase in size correspondingly. Because the plant cannot adjust itself to this abnormal condition, the tumor cells become shrunken and dry and the tumor itself dies.

This rather exhaustive introduction is submitted to make clear the connection between the earlier work on plant tumors with a polyploidizing chemical (colchicine) and that here presented, recording experiments in treating plant bacterial tumors with other chemicals claimed to be chromosome-doubling (6, 7, 8, 10, 13). In other words, if these other chromosome-doubling chemicals produce polyploidy for geneticists, would they not also induce an excessive number of chromosomes in tumor cells and thereby kill the tumors, as did one application of a 2 per cent solution of colchicine?

SUBSTANCES OTHER THAN COLCHICINE THAT CAUSE CHROMOSOME DOUBLING

The effect of some, perhaps all, of the other chromosome-doubling substances is similar to that induced by colchicine on wheat, rye, and other seedlings (11, 14). The roots of the treated seedlings thicken, and at the ends there are tumor-like swellings the cells of which show abnormal mitosis (5, 6, 9, 12, 14).

The chromosome-doubling substances used in these experiments were as follows: Acenaphthene, a-methylnaphthalene, a-nitronaphthalene, 3–5-dibromopyridine (15), and apiole (7). A commercial preparation known as "Santomerse," a few drops in water, was used as an emulsifying agent.

Experiments with Acenaphthene

When acenaphthene was mixed with lanolin and brushed on 12 French marigold and on 16 Paris daisy (*Chrysanthemum frutescens* L.) tumors 1 cm. or less in diameter, the treated tumors were all alive and growing at the end of a month and were the same size as the controls.

Acenaphthene, in a filtered, saturated water solution, was brushed for 2 successive days on 55 bacterial tumors, less than 1 cm. in diameter, on French marigold. Twenty days later the tumors were of the same size and appearance as those of the controls. Leaf galls of French marigold and stem tumors of Guinea-gold, a variety of African marigold (*Tagetes erecta* L.), also were brushed with a filtered, saturated water solution of acenaphthene. No change in the tumors followed.

Because it was thought that failure of acenaphthene to cause injury might be due to its slight solubility in water, other solvents were tried.

A number of solvents of acenaphthene were tested on plant tumors before applying that chemical to learn the possible reaction of the galls to the solvent. Several had a killing effect in 24 to 48 hours, and were discarded. Among these were morphalin, tetralin, and dimethyl phthalate. Those found most satisfactory in not affecting the tumors, or the stems in the vicinity of the tumors, were chlorinated naphthalene and dioxan.

Although not a solvent, glycerin was mixed with acenaphthene at the rate of 5 cc. to $\frac{1}{2}$ g. of acenaphthene. This paste-like mixture was spread thickly on the galls, in the hope that there would be a slow but definite penetration of the acenaphthalene into the cells. No injury occurred, nor was there any change in the bacterial tumors. Growth continued as in the controls.

Chlorinated naphthalene proved to be a solvent of acenaphthene, but there was neither killing nor inhibition of growth subsequent to application of a solution to tumors of various ages. Either the acenaphthene did not penetrate far enough into the tissue or the solvent neutralized its action. Some other factor may have been working, for a reaction was noted in the very young galls. Stimulation of growth of tumors occurred in 28 marigold and 21 brushed daisy galls, and these were definitely larger than those brushed with chlorinated naphthalene for controls or the galls left unbrushed for other controls. The stimulation occurred slowly, was not excessive, and no collapse of tumor cells followed. Stimulation was not observed in tumors that were 27 or more days old before treatment.

Besides increase in size over the control tumors, the galls brushed with acenaphthene chlorinated naphthalene continued both to grow and live longer than did the controls. Tests made with both daisy and marigold tumors in summer and fall gave like results.

Five, 10, 15 per cent, and full-strength solutions of dioxan[1] showed no harmful effect when brushed on both young and old daisy and marigold tumors. Because the tumors did not discolor and continued to grow vigorously, it was considered safe to use dioxan as a solvent.

Marigold galls of different ages were brushed with different percentages of acenaphthene dissolved in dioxan. It was thought that some combination of age of tumor and strength of acenaphthene might result in sufficient penetration of the chemical to stimulate rapid chromosome divisions, so that a

[1] Dioxan is the trade name for diethylene oxide.

collapse of young growing cells would finally occur. The action and effect of colchicine would thus be simulated. However, this did not happen with marigold tumors, regardless of their age. Solutions of acenaphthene in dioxan were used up to 10 per cent. In this experiment, 164 marigold tumors, 7, 11, 12, 14, 15, 16, 21, 22, and 34 days old, respectively, were brushed from 1 to 4 times. There were 91 controls for comparison.

Death did not occur when marigold tumors were brushed 4 successive days with a 3 per cent acenaphthene solution and the plants were kept in a moist chamber to prevent surface drying. In the moist chamber there was

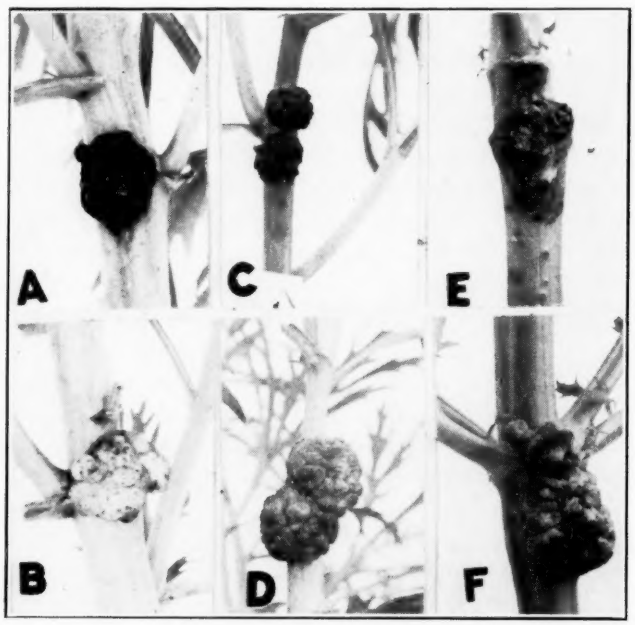

FIG. 1. A. Tumor on Paris daisy produced by inoculating with *Bacterium tumefaciens*, September 3, 1940; brushed with 3 per cent acenaphthene in dioxan, October 4, 1940; photographed October 10, 1940. B. Control of A. C. Paris daisy inoculated with *Bact. tumefaciens*, May 7, 1940; brushed with a-methylnaphthalene 50 per cent, June 14, 1940; Photographed, June 17, 1940. D. Control of C. E. Guinea gold marigold inoculated April 22, 1940; brushed with n-heptyl aldehyde 20 per cent May 17, 1940. Gall dead in three days, photographed June 7, 1940. F. Control of E. The brushed galls were dead when photographed, or died a few days later. All $\times 1$.

some penetration as indicated by shrinking and darkening of the tumors, but there were enough tumor cells alive and functioning to allow *Bacterium tumefaciens* to get a new start. This was shown by the development of outgrowths at the edges of the shrunken and darkened tumors. These rejuvenated tumors eventually became as large as the controls. Thirty-one treated marigold galls and 47 controls were studied under moist-chamber conditions.

Death occurred with daisy tumors, brushed with 3 per cent acenaphthene dissolved in dioxan, if the tumors were 36 or more days old and still growing. Tumors 31 days old or less did not all succumb. Some darkened,

some died (Fig. 1, A; control B), and the growth of others was inhibited. Out of 44 treated daisy tumors of different ages, 24 died.

A saturated water solution of the slightly soluble acenaphthene was used for watering marigold seedlings, later inoculated with *Bacterium tumefaciens* to learn whether or not the intake of this chemical would inhibit tumor production. Twenty-five marigold seedlings 2 to 2½ in. tall, grown in 3-in. pots, were watered daily for 10 days, each plant receiving 20–25 cc. of the solution. Sometimes crystals of acenaphthene were visible on the surface of the soil, but apparently without seedling injury. On the 10th day the 25 plants were inoculated, also 10 control plants. In 5 days the swellings at the points inoculated on the treated plants were larger than those on the control plants, a stimulation probably due to the chemical. The leaves were larger and had a broader horizontal spread in the treated plants than in the controls. This difference, however, lasted no longer than a week, after which the controls and treated plants appeared the same.

Four marigold and 4 daisy plants, 13 and 17 days old, respectively, bearing galls and growing in 6-in. pots were also watered with the acenaphthene-saturated water solution. The marigold galls were 5–7 mm. and the daisy galls 4–8 mm. in diameter. Each plant received 150 cc. of the solution per day for 10 days. Neither stimulation nor inhibition of the tumors on the treated plants was noted in comparison to the controls of the same age.

Haig Dermen examined free-hand sections of young daisy galls that had been brushed 3 times with 3 per cent acenaphthene dissolved in dioxan. The galls were first brushed when 16 days old and were examined 10 days later. In the interim they had received 2 other brushings with the same chemical. Dermen's statement is as follows: "Judging from 2 free-hand sections of young daisy galls, one treated with acenaphthene and the other untreated, the effect of acenaphthene appears to be comparable to that of weak colchicine (0.01 per cent solution) brushed on young marigold tumors." As it required a 1 to 2 per cent solution of colchicine to induce definite polyploidy in young daisy and marigold tumors, and ultimately kill them, the effect of the 0.01 per cent solution was slight; increase of nuclear and cell size occurred near the periphery (3) or in small areas (4).

Experiments with a-methylnaphthalene

A-methylnaphthalene in a saturated water solution, also a-nitronaphthalene and 3–5-dibromopyridine in saturated water solutions were all brushed twice on 12 French marigold and 16 daisy tumors, as had been done with acenaphthene. Lanolin, mixed with each of these chemicals, also was brushed on other marigold and daisy tumors. All results were negative.

Since a saturated water solution of a-methylnaphthalene had no effect when brushed on marigold or daisy tumors, the straight chemical and a 50 per cent emulsion were tested. Figure 1, C, shows a 38-day-old daisy tumor brushed once with a 50 per cent emulsion. The photograph was made 3 days after brushing. The tumor was dead in 5 days. A control tumor of the same age is shown in figure 1, D.

The full strength of a-methylnaphthalene was brushed once on marigold tumors 8, 17, 21, 28, 31, 43, and 44 days old, respectively, and from 4 mm. to 2½ cm. in diameter. The older tumors were still growing when treated. In all, 55 tumors were brushed and 38 were held as controls. Tumors of 20 days and older, darkened and died in from 2 to 7 days after brushing. Young 8-day-old galls did not shrink or darken under the treatment, but were more or less inhibited and grew to only half the size of the controls.

Forty-nine-day-old tumors on *Bryophyllum pinnatum* (Lam.) Kurz, white, sound, and growing well, showed no effect from the full-strength a-methylnaphthalene, while 23-day-old tumors on the closely related plant *Kalanchoe daigremontiana* (Ham. and Per.) Berger darkened in 1 day and were dead in 2 weeks. The vessels, however, through these dead tumors and the dead stem tissue occurring just above and below them, continued to function for some time, as shown by the fact that the other parts of the plants lived more than a month.

A-methylnaphthalene, full strength, was brushed once on 27 daisy tumors 3 mm. to 1¼ cm. in diameter, aged 7, 27, 30, 36, and 49 days, respectively. All were killed in 2 to 7 days, except those 7 days old. These darkened and ceased growing but did not die. Thirty-one tumors were kept unbrushed for controls.

Two brushings of a 50 per cent emulsion of a-methylnaphthalene acted much the same as one brushing of the full-strength chemical by killing the older tumors and inhibiting the younger ones. Seventy marigold tumors 9, 15, 27, 28, 33, 41, and 44 days old and 18 daisy tumors 9, 27, 38, and 49 days old were treated, while 50 and 24 controls, respectively, were held for comparison.

When 2 per cent and 10 per cent acenaphthene, respectively, were added to 50 per cent emulsions of a-methylnaphthalene and brushed on bacterial plant tumors, the results were essentially those of a-methylnaphthalene emulsion alone. Growth in young marigold and daisy tumors usually was retarded after brushing with emulsions containing either the 2 or the 10 per cent acenaphthene and the tumors either did not grow at all or were smaller than the controls. There were some cases where small tumors, 4 to 8 mm. in diameter, after retardation, finally reached the size of the controls, and occasional tumors of this size were not retarded at all.

Tumors 30 days old and older, 1 cm. and over in diameter, usually darkened immediately and died in 2 to 7 days.

Experiments with Apiole

Apiole is an oily, non-volatile compound extracted from parsley seed. It is one of the few plant extracts said to produce polyploidy (7). Its effect on bacterial plant tumors was unlike that of colchicine.

Twelve Paris daisies, inoculated 17 days, bearing tumors 4–8 mm. in diameter, were brushed with full-strength apiole. Twelve marigold tumors, 13 days old, and 5–7 mm. in diameter, also were brushed. No change took

place in the galls in 24 hours, so they were brushed again. In a month the treated tumors on both daisy and marigold were alive and had grown, but were smaller than the controls. Dwarfing followed the treatment but neither darkening nor death.

Fifteen marigold seedlings were watered with a 2 per cent apiole emulsion on 5 successive days, after which the stems were inoculated with *Bacterium tumefaciens*. The treated seedlings appeared less thrifty than the nontreated ones. That this difference in degree of vigor influenced the size of the galls is shown by the observation that a month after inoculation, the tumors on the apiole-treated plants were ¼ the size of those on the controls. About 3 weeks later the apiole-treated plants had sickened and died, but there was no prevention of tumor formation.

CHEMICALS NOT KNOWN TO CAUSE DOUBLING OF CHROMOSOME NUMBER

Two chemicals, normal heptyl aldehyde and methyl salicylate, not known to cause chromosome-doubling and used by animal pathologists in the control of mouse tumors, also were used in these experiments. With mouse tumors, growth is reported as slowed down; liquefaction, regression, and, even disappearance, of tumors following without affecting normal cells (16, 17).

Experiments with Methyl Salicylate

The tests consisted of (a) watering seedling marigold plants with emulsions of methyl salicylate to learn what effect this might have on tumor formation; (b) watering older marigold and daisy plants, having sizeable stem tumors, with emulsions of methyl salicylate; and (c) brushing tumors of different ages with methyl salicylate.

Marigold seedlings were watered for 5 successive days with different strengths of emulsions of methyl salicylate, after which the seedlings were inoculated with *Bacterium tumefaciens* to learn whether or not the formation of tumors would be prevented or perhaps delayed. The watering was continued for 12 days, each seedling receiving daily 20 to 25 cc. Of the 6 different strengths of emulsion tried, 0.3 per cent was the only one that did not kill the seedlings. One per cent, and higher, emulsions killed the seedlings in 1 to 2 days. In less than 2 weeks after inoculation tumors began to form on seedlings watered with 0.3 per cent emulsion. This was 5 to 6 days later than the controls, but the tumors soon reached the size of the controls.

Six large marigold plants, growing in 6-in. pots, with tumors 18 days old and 8 mm. to 1 cm. in diameter, were watered 18 times with 0.3 per cent emulsion of methyl salicylate. Twelve days after the watering began the tumors had become somewhat shrunken and slightly changed in color. No further change took place, even up to 22 days.

Six large Paris daisy plants with galls 21 days old, 5 mm. to 1 cm. in diameter, growing in 6-in. pots were watered with the 0.3 per cent emulsion at the same time as the marigold plants. Fifteen days after treatments

FIG. 2. A. *Bacterium tumefaciens* tumor on French marigold, 3 cm. in diameter, 44 days old and still growing when brushed, April 24, 1940, with methyl salicylate. Gall had blackened and shrunk some in one day when photographed, April 25, 1940; dead in 7 days. B. Control tumor on French marigold same age as A; inoculated with *Bact. tumefaciens*, March 11, 1940; photographed April 25, 1940. Both $\times 1$.

started the galls began to shrink and darken; in 18 days they had become dry and dark; and in 24 they were dying. In 31 days the plants, themselves, began to show effects of the treatment by a yellowing of the leaves. At this time the control tumors were still growing.

Brushing young marigold tumors, 12 to 19 days old once with full strength of methyl salicylate, did not kill them. It retarded their growth for a time, but later they recovered and reached their full development. When marigold tumors were 30 to 44 days old, but still growing, they became shrunken, turned black, and died in less than a week (Fig. 2, A; control 2, B). French marigold tumors succumbed more readily than did those of the Guinea-gold marigold. Usually, if the tumors were larger than 1 cm. across, they died. The stems of treated plants were uninjured.

Marigold tumors 11 days old, 5 to 9 mm. in diameter, died subsequent to brushing 3 times at intervals of 1 day between brushings. This was a very severe treatment. The plants in this series brushed once remained alive and continued to grow.

Daisy tumors were far more sensitive to the full-strength methyl salicylate than were the marigold tumors. Some of them shrank nearly to half size, while the plant remained healthy. Daisy tumors 16 days old, 8 mm. to 1¼ cm. in diameter, and daisy galls 26 days old, 1½ to 2½ cm. in diameter, and still growing, were all dead 10 days after brushing. Very young daisy tumors 7 days old, 3–4 mm. in diameter, were inhibited in growth and became slightly darkened but did not die.

Full-strength methyl salicylate brushed on *Kalanchoe diagremontiana* tumors 2 months old, killed them but did not kill *Bryophyllum pinnatum* tumors of the same age. The Kalanchoe controls were alive and growing 4 to 5 months later, while the brushed ones were dead in less than 3 weeks. The treated Bryophyllum tumors were alive and growing after 5 months.

Experiments with N-heptyl Aldehyde

Twenty-five marigold seedlings were watered with n-heptyl aldehyde, each of 5 plants receiving 1,000, 700, 500, 200, and 100 parts per million, respectively. At the seventh watering the plants were inoculated with *Bacterium tumefaciens*. Thirteen days after commencing the experiment the treated seedlings were larger and more vigorous than the 25 nontreated controls. Nine days after inoculation, the galls on the treated seedlings were as large as those on the controls, and continued to grow equally well.

Other marigold seedlings were watered with 2 per cent, 1, ½, ¼, and ⅛ per cent n-heptyl aldehyde. These seedlings were able to withstand the ¼ and ⅛ per cent emulsions, and a few were not killed by the ½ per cent emulsion. Most of those watered with the 2, and 1 per cent emulsions died. After watering with the ¼ and ⅛ per cent emulsions, respectively, for 5 successive days, the plants were inoculated with *Bacterium tumefaciens* and the watering continued 5 more days. Tumors formed on the seedlings and grew as well as on the controls.

Brushing 25-day-old marigold tumors 1 to 1½ cm. in diameter on 2 successive days with a 5 per cent emulsion of n-heptyl aldehyde was without effect. When 18-day-old marigold tumors, 5–7 mm. in diameter, were brushed with a 10 per cent emulsion there was some darkening from which the tumors recovered. Other marigold galls, 20 days old, brushed with the 10 per cent emulsion, became darkened for a time but recovered later. However, 1 brushing of a 20 per cent emulsion killed 12 marigold tumors 25 days old in 3 days (Fig. 1, E; control 1, F). Eight other marigold tumors 25 days old, treated with the 20 per cent emulsion mixed with bentonite, also were dead after 3 days. In addition, seven 35-day-old marigold tumors blackened and died in 3 days after 1 brushing with this mixture. There was a slight stem injury just above and below the tumors with the 20 per cent emulsion. Young marigold tumors 8 days old and 4–5 mm. in diameter were not affected by 1 brushing of the 20 per cent emulsion. No change occurred in the 12 galls in a week, and, in 10 days, they were nearly the size of the controls.

Full-strength n-heptyl aldehyde produced a rapid toxic effect on 12 marigold tumors 14 days old. Four hours after brushing, the tumors were shrunken and the stems darkened on either side of the outgrowth. In 24 hours the stems of 8 of the 12 plants had collapsed and bent over at the site brushed, and the tumors were dead.

Paris daisy tumors, 18 days old, brushed with full-strength n-heptyl aldehyde, were not affected in 24 hours as were the tumors on marigold plants, nor did the daisy stems bend over as did the marigold stems. In that time the daisy tumors had shrunk a little and a few leaves had dropped. In 2 days all 12 of the daisy tumors were shrunken and beginning to dry. In 11 days the tumors were alive and had grown some, but in 18 days 6 out of the 12 were dead. The other 6 continued slowly to increase in size.

Daisy tumors, 46 days old, varying in size from 8 mm. to 1½ cm., were brushed with a 10 per cent n-heptyl aldehyde emulsion. In 3 days 6 of the 10 tumors brushed had shrunk and darkened and the leaf petioles near the galls also had darkened. In 10 days all the galls were blackened but still alive. In a month, however, they were dead, while the plants were still in very good condition.

Methyl salicylate and n-heptyl aldehyde seemed to have a toxic effect on both marigold and daisy tumors, according to the age and growing condition of the tumors and the strength of the emulsion used. In most cases young tumors were less susceptible to the chemicals than the older ones.

DISCUSSION

The results with acenaphthene in experiments with plant tumors were unlike those with colchicine. With the latter, there was stimulation of cell growth followed by cell collapse and death. Marigold tumors were practically unaffected by acenaphthene dissolved in dioxan, regardless of age or growing condition, while the daisy tumors, if not too young, became shrunken

and blackened. Young daisy galls were unaffected. Out of a total of 164 marigold tumors brushed with acenaphthene dissolved in dioxan not one died. Any slight inhibition of growth that took place was overcome in a short time and the treated tumors soon reached the size of the controls.

Of the 44 daisy tumors brushed with acenaphthene dissolved in dioxan, 24 died. The other 20 lived and in most cases eventually reached the size of the controls. In no case did a young daisy tumor brushed with this solution show more than a slight darkening; indeed, those brushed when 31 days old, although at first somewhat shrunken and darkened by the treatment, entirely recovered.

It seems that the slowly penetrating acenaphthene is taken care of in a young growing plant by dispersion throughout its system. This gives a chance for the tumor to recover and continue its growth. Dispersion does not take place in the case of older daisy tumors. There were some differences in the reaction of tumors to the other chromosome-doubling chemicals, but, in the main, the action of these chemicals closely resembled that of acenaphthene.

According to Dermen's findings, the nuclei of young plant tumors seem not to be very much affected by acenaphthene, either because of a lack of penetration or from other causes. It is possible that death of older tumors may occur because there is not enough active growth for a systemic absorption of the chemical by the plant and a lytic effect follows because of its concentration in the tumors.

SUMMARY

The chromosome-doubling chemicals acenaphthene, a-methylnaphthalene, a-nitronaphthalene, 3–5-dibromopyridine, and apiole did not act on bacterial plant tumors produced by *Bacterium tumefaciens* in the same way as colchicine. The plant-tumor cells seemed to have their own specific response to the given chemical according to the species on which the tumor grew and, with a few exceptions, according to the age of the tumor. No excessive doubling of chromosomes in the nuclei of young tumors treated with these chemicals took place as did in young tumor nuclei treated with colchicine.

There was no stimulation of tumor growth by acenaphthene, except in those plant tumors brushed with this chemical dissolved in chlorinated-naphthalene and temporarily in those seedling marigolds watered with a saturated water solution of acenaphthene.

Acenaphthene dissolved in dioxan did not produce the noticeable stimulation of tumor development so characteristic of colchicine before inhibition and death take place. Three per cent acenaphthene in dioxan did not kill marigold tumors, young or old; it killed Paris daisy tumors 36 or more days old but not young daisy tumors.

Any inhibition of growth of marigold tumors by the action of acenaphthene dissolved in dioxan was usually overcome, and the treated tumors on reaching maturity were the size of the controls at maturity.

TABLE 1.—*Summary of reaction of crown gall to application of various chemicals*

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
3% Acenaphthene in dioxan[a]	French marigold	1 to 1¼ cm.	21	21	3	7	33	In 2 to 4 days after brushing, the tumors had darkened and shrunk a little. In 10 days they had recovered and were like control tumors.
" "	" "	¾ to 1 cm.	19	15	3	7	35	" "
" "	" "	4 to 5 mm.	5	11	4	5	22	" "
" "	" "	3 to 5 mm.	7	11	4	4	47	" "
" "	" "	3 to 4 mm.	6	7	3	6	12	" "
" "	" "	4 to 5 mm.	7	48	2	7	12	" "
" "	Paris daisy	1 to 1½ cm.	21	36	1	7	12	16 of the 21 tumors dead.
" "	" "	7 to 9 mm.	7	31	1	7	21	2 tumors dead; others alive.
" "	" "	1 to 1½ cm.	7	41	1	7	13	All tumors dead.
" "	" "	4 to 5 mm.	6	18	2	6	17	Young tumors not killed: were nearly as large as the controls.
3% Acenaphthene in dioxan	Paris daisy	1¼ to 1½ cm.	3	48	1	2	10	One tumor dead; the others growing.
5% Acenaphthene in dioxan	French marigold	1 to 1¼ cm.	7	22	4	7	37	Darkened and shrunk the first few days, then recovered and were like the control tumors.
" " "	" "	¾ to 1 cm.	28	16	4	14	26	" "
10% " "	" "	1 to 1¼ cm.	7	22	4	7	28	" "
" " "	" "	¾ to 1 cm.	14	16	3	14	26	" "
" " "	" "	4 to 5 mm.	5	11	4	5	22	" "

[a] To test the effect of moist chamber conditions, 31 other French marigolds with tumors of similar sizes and ages were treated with 3–5–10 per cent acenaphthene in dioxan. Results were the same as on those tumors kept in the open greenhouse.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
1% Acenaphthene in lanolin	French marigold	1½ to 2 cm.	12	28	1	12 in pure lanolin	34	None
" " "	" "	¾ to 1 cm.	6	18	1	6 in lanolin	34	None
3% " "	" "	¾ to 1 cm.	12	15	1	7 in lanolin	30	"
Acenaphthene in filtered saturated water solution	" "	¾ to 1 cm.	55	16	2	7	20	"
" "	French marigold seedlings, 2½ inches tall	———	—	—	25 received chemical in soil 10 times, then inoculated with *Bacterium tumefaciens*	10	19	Tumors showed stimulation over controls for a week after forming; later they were same size as the controls.
5% Acenaphthene in chlorinated naphthalene	" "	¾ to 1½ cm.	28	22	2	10	20	Stimulation of tumor growth in those under 27 days old when treated.
" "	Paris daisy	8 mm. to 1 cm.	21	25	2	10	39	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
1% 3–5 dibromopyridine in lanolin	French marigold	6 to 9 mm.	12	18	1	12 with pure lanolin	34	None
3–5 dibromopyridine in filtered saturated water solution	" "	1½ to 2 cm.	12	27	2	6	34	"
" "	Paris daisy	3 to 8 mm.	16	19	2	6	36	"
" "	" "	½ to 1 cm.	6	23	2	6	22	"
" "	Guinea gold marigold	1 to 1½ cm.	6	19	2	6	22	"
1% a-nitronaphthalene in lanolin	French marigold	1½ to 2 cm.	12	28	1	12 with pure lanolin	32	"
a-nitronaphthalene in saturated water solution	" "	6 to 9 mm.	12	18	2	6	34	"
" "	Paris daisy	3 to 8 mm.	6	19	2	6	36	"
" "	" "	1½ to 1 cm.	5	23	2	5	22	"
" "	Guinea gold marigold	1 to 1½ cm.	6	19	2	6	22	"
1% a-Methyl naphthalene in lanolin	French marigold	1½ to 2 cm.	12	28	2	12 with lanolin	34	"
a-Methyl naphthalene saturated water solution	French marigold	1 to 1¼ cm.	12	19	2	6	32	None
" "	Paris daisy	3 to 8 mm.	16	19	2	6	36	"

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
a-Methylnaphthalene 50% emulsion	French marigold	1¾ to 2½ cm.	3	44	1	6	14	Tumors dark and shrunk in 2 days; dead.
" "	" "	1½ to 2½ cm.	7	41	2	7	21	Dark in 1 day; dead.
" "	" "	¾ to 1¼ cm.	21	33	7 brushed 2 times	7	24	Dark in one day; 3 dead; those brushed twice were half the size of those brushed once.
" "	" "	¾ to 1¼ cm.	3	28	1	6	27	Tumors dark in one day; half of them dead later, others barely alive.
" "	" "	¾ to 1 cm.	12	15	6 brushed 2 times	12	30	Growth inhibited at once in those brushed twice; were ¾ size of control tumor in a month. None dead.
" "	" "	4 to 5 mm.	12	9	1	6	27	Tumors nearly size of controls.
" "	Paris daisy	1¼ to 2 cm.	3	49	1	6	2	Tumors dark in one day; dead in 2 days.
" "	" "	1 to 1½ cm.	6	38	1	6	5	Tumors dark and shrunk in one day; dead.
" "	" "	6 to 10 mm.	3	27	1	6	7	Tumors dark in one day; dead.
" "	" "	4 to 5 mm.	6	9	1	6	41	Tumors slightly dark in one day; were inhibited, but alive.
a-Methylnaphthalene full strength	French marigold	¾ to 2½ cm.	3	44	1	6	7	Dead.
" "	" "	1½ to 2 cm.	5	43	1	5	5	Dead.
" "	" "	1 to 2 cm.	12	31	1	6	4	Dead.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
a-Methylnaphthalene full strength	French marigold	1¼ to 1½ cm.	3	28	1	6	7	Dead.
" "	" "	¾ to 1¼ cm.	7	21	1	5	4	Dead.
" "	" "	4 to 8 mm.	18	17	1	5	28	Tumors browned in 2 days but later recovered and grew to size of controls.
" "	" "	4 to 5 mm.	7	8	1	5	30	Tumors were dark and smaller than control tumors.
" "	*Bryophyllum pinnatum*	5 mm. to 1 cm.	6	49	1	4	30	No effect on tumors.
" "	*Kalanchoe daigremontiana*	¾ to 1 cm.	6	23	1	4	14	Tumors darkened in one day; dead in 2 weeks.
" "	Paris daisy	1½ to 2 cm.	3	49	1	6	2	Tumors dead.
" "	" "	1 to 1¼ cm.	6	36	1	7	7	" "
" "	" "	¾ to 1¼ cm.	9	30	1	5	4	" "
" "	" "	6 to 10 mm.	3	27	1	6	2	" "
" "	" "	3 to 4 mm.	6	7	1	7	21	Tumors inhibited.
Apiole 2% emulsion	French marigold seedlings	Inoculated after treating	—	—	Emulsion on seedlings for 5 days	10	35	Treated seedlings were inoculated with *Bact. tumefaciens* after 5 treatments. Tumors formed; ¼ size of control tumors.
Apiole, full strength	Guinea gold marigold	5 to 7 mm.	12	13	Brushed 2 times	6	42	Slowing down of tumor formation and dwarfing.
" "	Paris daisy	4 to 8 mm.	12	17	2	6	46	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
Methyl salicylate 0.3% emulsion	French marigold 10 seedlings	Inoculation after treating	—	—	Watered seedlings with emulsion 12 days	20	21	Tumors formed 5 to 6 days later on treated seedlings than on controls. Reached control tumor size later.
" "	French marigold plants	8 mm. to 1 cm.	6	18	Watered with emulsion 18 times	3	22	Tumors had darkened and shrunken some in 12 days. No further change.
" "	Paris daisy plants	½ to 1 cm.	6	21	" "	3	24	Tumors dying in 24 days.
Methyl salicylate full strength	French marigold	1 to 1¾ cm.	12	24	Brushed 2 times	6	24	Tumors dead.
" "	" "	½ to 1¼ cm.	8	19	1	6	48	Tumors alive.
" "	" "	3 to 3½ cm.	3	44	1	3	6	Tumors dead.
" "	" "	2 to 2½ cm.	3	30	1	3	6	" "
" "	" "	1 to 1¼ cm.	6	25	1	4	22	" "
" "	Guinea gold marigold	8 to 10 mm.	6	14	2	6	35	Three tumors dead, others inhibited.
" "	" "	5 to 7 mm.	18	15	3	7	17	Tumors dead.
" "	" "	5 to 8 mm.	9	19	1	7	18	Tumors alive and growing.
" "	" "	5 to 9 mm.	12	11	1	7	17	Tumors alive; darkened.
" "	" "	5 to 9 mm.	6	11	3	7	17	" dead.
" "	" "	2 to 2½ cm.	6	57	1	3	2	" "
" "	" "	6 mm. to 1 cm.	24	23	1	6	16	" darkened but growing.
" "	" "	6 mm. to 1 cm.	6	23	2	6	8	" dying.
" "	" "	6 mm. to 1 cm.	6	23	3	6	8	" dead.
" "	" "	6 mm. to 1 cm.	6	25	4	6	8	" "
" "	Paris daisy	3 to 4 mm.	5	7	1	3	33	" darkened and inhibited.

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			Number	*Days*	*No. times brushed*	*No.*	*Days*	
Methyl salicylate full strength	Paris daisy	6 to 8 mm.	6	18	1	6	19	5 tumors dead, one alive.
" "	" "	8 mm. to 1¼ cm.	3	16	1	3	10	Tumors dead.
" "	" "	1 to 1¼ cm.	3	36	1	3	2	" "
" "	" "	1½ to 2½ cm.	6	26	1	6	10	" "
" "	*Kalanchoe daigremontiana*	1½ to 2 cm.	3	58	1	3	20	" "
" "	*Bryophyllum pinnatum*	½ to 1 cm.	3	60	1	3	150	" alive and growing.
n-heptyl aldehyde 1000 parts per million	French marigold seedlings	Inoculated after treating	—	—	7 seedlings treated 7 times, then inoculated	25	18	Treated seedlings larger than control seedlings. No checking of tumor growth.
n-heptyl aldehyde 700 parts per million	French marigold	Inoculated after treating	—	—	7 seedlings treated 7 times, then inoculated	25	18	Treated seedlings larger than control seedlings. No checking of tumor growth.
n-heptyl aldehyde 500 parts per million	" "	" "	—	—	" "	25	18	" "
n-heptyl aldehyde 200 parts per million	" "	" "	—	—	" "	25	18	" "
n-heptyl aldehyde ¼% emulsion	" "	" "	—	—	Treated 10 times. Inoc. after 5 treatments	5	22	" "
n-heptyl aldehyde ⅛% emulsion	" "	" "	—	—	" "	5	22	" "

TABLE 1.—(*Continued*)

Chemical amount and solvent	Host bearing crown gall				Application		Results	
	Kind of plant	Size of tumor	Tumors treated	Age of tumor when treated	Manner of treatment	Controls	After	Reaction
			No.	*Days*	*No. times brushed*	*No.*	*Days*	
n-heptyl aldehyde 5% emulsion	French marigold large plants	Tumors 1 to 1½ cm.	8	25	Brushed 2 times	4	12	No inhibition of tumor growth.
n-heptyl aldehyde 10% emulsion	Guinea gold marigold	5 to 7 mm.	18	15	2	12	24	" " " " "
" "	" "	5 to 9 mm.	12	20	3	12	22	" " " " "
" "	Paris daisy	8 mm. to 1¼ cm.	10	46	1	5	24	Tumors dead.
n-heptyl aldehyde 20% emulsion	Guinea gold marigold	1 to 1¼ cm.	12	25	1	6	20	" "
" "	" "	1½ to 2¼ cm.	7	35	1	6	13	" "
" "	French marigold	4 to 5 mm.	12	8	1	6	40	Tumors not inhibited or killed; too young to be affected.
n-heptyl aldehyde in bentonite	Guinea gold marigold	1 to 1¼ cm.	8	25	1	6	20	All tumors killed.
n-heptyl aldehyde full strength	" "	5 to 7 mm.	12	14	1	6	5	All tumors dead.
" "	Paris daisy	4 to 8 mm.	12	18	1	6	12	Tumors alive but ⅓ size of control tumors.

Dwarfing occurred when apiole was brushed on tumors, but there was no darkening or death.

Full-strength a-methylnaphthalene brushed on young marigold and Paris daisy tumors inhibited further growth; it killed old but growing tumors on both hosts. A 50 per cent emulsion had much the same effect. The two related plants *Bryophyllum pinnatum* and *Kalanchoe daigremontiana* responded differently to a a-methylnaphthalene brushed on stem tumors. In a month there was no effect on Bryophyllum, while Kalanchoe tumors were dead in 2 weeks.

A-nitronaphthalene and 3–5-dibromopyridine were used only in saturated water solutions and in lanolin paste. Young and old Paris daisy and marigold tumors were brushed with these chemicals, with negative results. Two chemicals, heptyl aldehyde and methyl salicylate, not known to be polyploidizing agents, but used by animal pathologists in the control of mouse tumors, were also applied to bacterial plant tumors. Brushing normal heptyl aldehyde full strength on 12 marigold tumors, 14 days old, killed them in 24 hours, while 18-day-old daisy tumors became slightly shrunken. In 3 weeks only half of the 18-day-old tumors were dead; the other 6 tumors continued to live and grow. A 20 per cent emulsion of n-heptyl aldehyde killed 20 marigold tumors, 25 days old, in 3 days; 8-day-old marigold tumors were not affected. A 10 per cent emulsion of this chemical killed 10 46-day-old daisy tumors.

Full strength a-methyl salicylate killed 30- to 44-day-old marigold tumors, but not 12- to 15-day-old marigold tumors. Paris daisy tumors, 16 to 26 days old, were killed with a-methyl salicylate, and tumors 7 days old when brushed were inhibited.

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EYE-SPOT OF NAPIER GRASS IN HAWAII, CAUSED BY HELMINTHOSPORIUM SACCHARI[1]

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INTRODUCTION

Napier grass (*Pennisetum purpureum* Schum.) was first introduced into Hawaii in 1912 from Africa (31); since that time it has become highly esteemed by local ranchers as a soiling crop and for pastures. In October, 1939, specimens of diseased Napier, or elephant grass as it is also called, which showed lesions on the leaves, leaf sheaths, and stems, together with a general killing of the plant, were received from Kona, on the Island of Hawaii. Since the original outbreak, the disease has spread to all of the islands of the Hawaiian group, causing appreciable losses. In the present paper facts concerning the disease and its causal agent *Helminthosporium sacchari* (van Breda de Haan) Butler, are presented.

SUSCEPTS

Plants Affected

Napier grass and sugar cane (*Saccharum officinarum*) are the plants most commonly affected by this disease, but Mitra (20) has reported that the leaves of wheat, barley, oats, maize, sorghum, rice, and *Pennisetum typhoideum* are also susceptible. The writer has demonstrated that *P. setosum* is not susceptible, and Paterson (24) has reported that Guatemala grass (*Tripsacum laxum*), growing alongside severely diseased Napier, showed no symptoms of disease.

Varietal Susceptibility

Stokes and Ritchey (28) report that resistant and immune strains of Napier grass are known in Florida. Voorhees (29) inoculated a number of these strains and found that none of the so-called "immune" strains became infected, whereas all strains originating from susceptible Napier developed the disease. Merker grass, a variety of Napier (30) and several reciprocal Napier × Merker crosses developed by the Hawaii Agricultural Experiment Station are resistant to the disease (22).

THE DISEASE

Name

The name "eye-spot" was assigned to this disease of Napier grass by Voorhees (29), who believed that it was caused by the fungus responsible for eye-spot of sugar cane. The writer concurs in this opinion, and the name eye-spot is retained and used throughout this paper to denote the disease of Napier as it occurs in Hawaii.

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History and Range

Paterson (24) probably is referring to eye-spot when he describes a disease of Napier in Trinidad in 1933, which produced elongated, reddish-brown spots on the leaves. Ultimately the leaves were killed and the plant was checked in its growth. Stell (26) also records eye-spot on Napier in Trinidad; adjacent sugar cane was unaffected. In 1935, Leukel and Camp (15) mention that yields of Napier in Florida were severely reduced by a disease, which Stokes and Ritchey (28) and Voorhees (29) later demonstrated was eye-spot. The disease probably occurs in other parts of the tropics on Napier, but no further references have been found in the literature.

In Hawaii eye-spot undoubtedly was present some months prior to its discovery, for the first specimens examined were in advanced stages of the disease. According to statements by ranchers of long standing in the Kona community, many years ago a similar outbreak occurred and fields of Napier were destroyed; later, the disease disappeared. Whether or not the disease in question was eye-spot is uncertain, but it is significant to note that Paterson (24) records that sudden fluctuations in the severity of eye-spot are not uncommon.

Importance

This disease may cause losses in any one or all of several ways. The leaves may be killed prematurely or be so badly affected that they dry out and become unpalatable. On the stems, cankers are formed which interfere with normal physiological activities of the grass and cause premature drying and shedding of foliage. Cankered stems quickly become pithy and hollow and unfit for stock feed. Young buds, at or near the soil level, may be weakened or killed outright. Secondary organisms enter the rootstock by way of these dying or dead buds, and initiate decay.

Losses due to this disease are undoubtedly of appreciable magnitude, but no monetary estimate has been made. Where the disease has appeared in severe form, entire plantings have been rendered useless for fodder, and many have been plowed and replanted with other grasses.

Symptomatology

Morphologic Symptoms on the Leaves. Initial spots appear as small, yellowed areas within normal green tissues; later, the spots turn reddish-brown and may exhibit a yellow halo. The center of the spots may be lighter brown than the periphery, and sometimes one or more rings are present to give a zoning effect. In shape, the spots are oval and fairly regular in outline except where two or more coalesce to produce an irregularly shaped lesion (Fig. 1, A). They measure 1 to 6 mm. long by 0.5 to 2 mm. wide, with the greater dimension always parallel to the long axis of the leaf, and are very similar to spots described by Voorhees (29).

Runners or streaks may be present on diseased leaves (Fig. 1, B), extending from the primary lesion for several inches toward the apex of the

leaf. Their color is essentially the same as that of old lesions, and the tissues involved are obviously dead. Compared to the damage produced on sugar cane in Hawaii by eye-spot runners (16), the damage on Napier caused by runners is slight. Streaks of dead tissue not associated with primary lesions, accompanied by anthocyanescence, may also be produced on

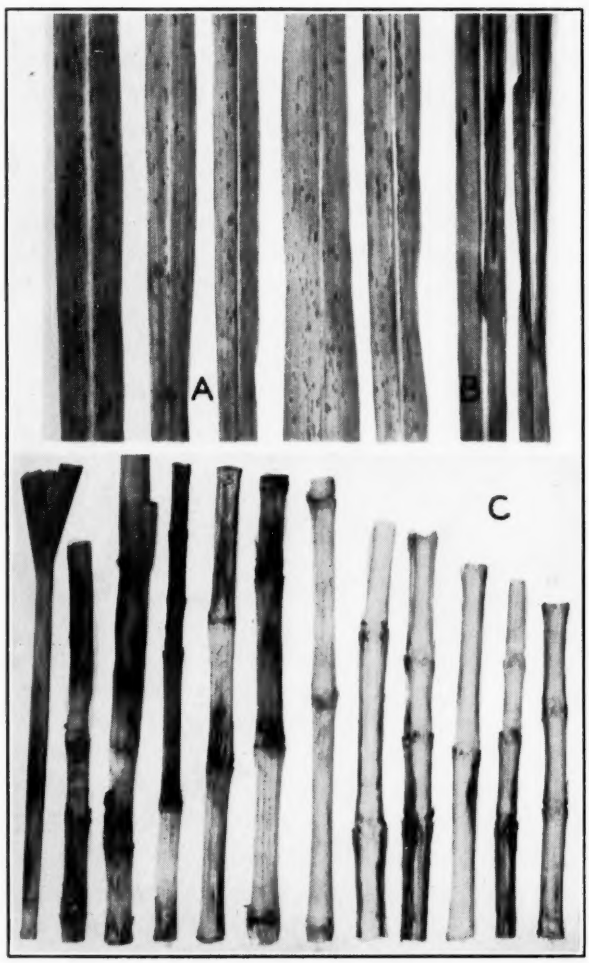

FIG. 1. Eye-spot caused by *Helminthosporium sacchari*, on Napier grass. A and B. On leaves; in B, runners are shown. C. On leaf sheaths and stems; note cankers, external and internal, and withering of stem. Healthy stem in center.

the leaves. These streaks usually are found on plants with cankered stems and probably are due to disturbance of the normal functioning of the vascular system of the stem by the pathogen.

The effect of the disease is more pronounced on old leaves than on young, and the growing tip is seldom attacked. This is in opposition to findings by Martin (16) and others in Hawaii for the effect of eye-spot on susceptible varieties of sugar cane. Severe infection causes the leaves to wither,

dry out, and die prematurely in acropetal succession: eventually, most of the leaves hang pendent or fall to the ground and the stem is bare except for a tuft of three or four leaves.

On the Leaf Sheaths. Lesions are commonly found on the leaf sheaths (Fig. 1, C). They are larger than spots on the leaves, lighter in color, and more diffuse in outline. Penetration of the sheath may take place and the stem itself be attacked.

On the Stems. Stem lesions are more elongate than leaf lesions and correspondingly narrower (Fig. 1, C). They are smooth at first, regular in outline and reddish-brown, with or without a lighter-colored center; later they become sunken, irregular in shape, and bluish-purple to black; the border may be grayish-white or retain the original reddish-brown color. Lesions are found from ground level to several feet above the soil, but usually occur on the first 6 inches of the stem. The nodes are more commonly attacked than the internodes. Affected stems are shrunken, pithy, and partly or completely hollow; when split longitudinally in the region of cankers, the interior of the stem is seen to be diseased or dead in part or in whole (Fig. 1, C).

On the Crown. The crown is seldom affected; occasionally, however, diseased tissue can be found and the basal buds are withered and darkened and their subsequent growth is stunted. The roots are not thought to be attacked, and Voorhees (29) mentions no symptoms on these parts. However, plants affected with eye-spot have weakened root systems and are quickly knocked over and trampled by grazing animals. *Marasmius* sp. (*sacchari*?) is present in decayed Napier roots; this fungus possibly attacks plants debilitated by eye-spot.

On the Plant as a Whole. From a distance, badly diseased Napier appears to be blasted by lightning or burned by fire. Plantings that have been diseased for some time show an abundance of partly developed adventitious side shoots, in various stages of destruction by the disease.

ETIOLOGY

Name, History, and Classification of the Pathogen

The organism causing eye-spot of Napier in Hawaii is believed to be the same as that producing eye-spot of Napier in Florida, which Stokes and Ritchey (28) and Voorhees (29) state is *Helminthosporium ocellum* Faris. This fungus was claimed by Faris (9), whose data were accepted by Bourne (2), to be the cause of eye-spot of sugar cane in Florida, Cuba, Santo Domingo, Puerto Rico, and Hawaii. For many years previously, sugar-cane pathologists had ascribed eye-spot to *H. sacchari* Butler (4), spoken of in India, from which it was originally described in 1913, as the cause of "helminthosporiose." Faris' arguments in favor of the erection of a new species were based on a difference in symptom expression and spore length. *H. sacchari* was reported to occur equally on the midrib and the thin part of the leaf and to have a mean spore length of 47.5 μ, while Faris found that

H. ocellum occurred more frequently on the thin part of the leaf than elsewhere, and the mean spore length was 69 μ. Investigators prior to Faris had similarly found that the spore length reported by Butler was unusually low and that *H. sacchari,* or *Cercospora sacchari* van Breda de Haan (3), as it was incorrectly named, produced spores that varied in mean length from 61 μ to 78 μ, depending on the observer (Table 1).

Mitra (20), working in India, has more recently shown that Butler's spore measurements do not indicate the full range of variability of *H. sacchari,* and that spore length, color, and curvature are determined by temperature and by the substrate on which the fungus is grown. Instead of 47.5 μ as the mean spore length, the figure should be advanced to 68.3 μ, which is almost identical with what Faris found. In a second paper Mitra (21) concludes that *H. sacchari* and its strains (saltants) have a range of variation in spore dimensions sufficiently wide to include all forms of *Helminthosporium* causing eye-spot.

McRae (19), also working in India, made a comparative study of the physiology of *H. sacchari, H. ocellum, H. stenospilum* Drechsler,[2] now known as *Cochliobolus stenospilus* (Carp.) M. and Y., and the unidentified species of *Helminthosporium* of Priode (25). The last 3 species were obtained from Florida, the *H. sacchari* from India. McRae's findings can be summarized as follows: (a) On certain media the species were much alike and appeared to be closely allied, while on other media they differed in certain cultural characteristics; (b) the range of spore measurements of the species and their strains under standard conditions fell within the range of *H. sacchari* and its saltants; (c) *H. sacchari* and *H. ocellum* were much alike, and from their growth in culture, spore length, and septation appeared to be allied; and (d) the type and color of lesions produced on cane leaves by the species and their saltants differed so slightly from those of each other that it was almost impossible to distinguish the fungi by their spot characters. McRae considered *H. ocellum* to be identical with *H. sacchari* but a different strain and recommended that the name *H. sacchari* Butler, with its amplified description by Mitra (20), be maintained for

[2] McRae (19) found one mutant of *Helminthosporium sacchari,* which produced spores similar in size and septation to spores of *H. stenospilum;* Drechsler (8) separated the two on the basis of the larger spores of *H. stenospilum* and a difference in symptoms with *H. stenospilum* causing brown stripe. Martin (16) reports that measurements of the spores of the brown-stripe fungus in Hawaii demonstrated that it was difficult to distinguish brown stripe from eye-spot. The writer isolated *H. stenospilum* from authentic cane material supplied by J. P. Martin, and measured the spores produced in culture. The following results were obtained:

At 21° C., on corn-meal agar:	25.6–73.6 μ (weighted mean 46.1 μ) by 9.6–18.4 μ (weighted mean 13.0 μ) with 3–9 septa per spore (weighted mean 5.4).
Ditto, on 1-per cent-sucrose agar:	32.0–76.8 μ (weighted mean 51.3 μ) by 12.0–20.0 μ (weighted mean 14.9 μ) with 3–8 septa per spore (weighted mean 5.8).
Ditto, nutrient agar:	no spores produced after one month.

At 28° C., no spores were produced on any one of above agars, after one month.

In color and shape, the spores of *H. stenospilum* closely resembled those of *H. sacchari* and their sizes were not unlike. This corroborates Martin's (16) previous rather unorthodox findings.

TABLE 1.—*Length, width, septation, color, and shape of spores of H. sacchari as found by various investigators*

TABLE 1.—*Length, width, septation, color, and shape of spores of H. sacchari as found by various investigators*

Investigator	Name assigned to pathogen	Length in μ			Width in μ			Septa			Color and shape
		Range	Mean	Weighted mean	Range	Mean	Weighted mean	Range	Mean	Weighted mean	
Kruger	*C. sacchari*	32.4– 90	.....	78.5	9.0–14.4	12.5	.....	5– 8	6.5	.....	Smoky-brown
Breda de Haan	*C. sacchari*	60.0– 80	70.0	.....	9.0–12.0	10.5	.....	5– 8	6.5	.....	Reddish-brown, lengthened oval
Cobb	*C. sacchari*	50.0– 72[a]	61.0[a]	.....	11.0–14.0[a]	12.5[a]	.....	3– 9[a]	.....	7.0	Dark, curved
Larsen	*C. sacchari*	53.0– 82	67.5	.....	9.0–15.0	12.0	.....	4–10	8.0	.....	Dark, curved
Butler	*H. sacchari*	35.0– 60	47.5	.....	8.5–12.0	10.2	.....	3–10	6.5	.....	Olive-green to brown, cylindrical or long elliptical
Cook (1924)	*H. sacchari*	45.0–110	77.5	.....	.....	12.0	.....	5–11	8.0	.....	.....
Cook (1924)	*H. sacchari*	30.0– 95	62.5	.....	12.0–15.0	13.5	.....	4–10	7.0	.....	.....
Cook (1927)	*H. sacchari*	22.0– 92	.....	58.7	6.6–15.0	.....	11.1	3–11	7.0	.....	.....
Johnson and Stevenson	*H. sacchari*	32.0– 90	61.0	.....	9.0–14.0	11.5	.....	several	.....	.....	.....
Bourne (1922)	*H. sacchari*	Up to 75	.....	.....	.....	.....	.....	.....	.....	.....	.....
Bourne (1934)	*H. ocellum*	24.0– 92	.....	58.7	11.1–16.7	.....	14.0	3– 9	.....	6.7	Light smoky yellow-brown, slightly curved
Faris (1928)	*H. ocellum*	32.0–110	.....	69.0	6.6–20.8	.....	12.7	3–10	6.5	.....	Light smoky yellow-brown, slightly curved
Faris (1928)	*H. ocellum*	29.0– 94	.....	69.0	9.0–21.0	.....	12.7	3–10	.....	6.7	Light smoky yellow-brown, slightly curved
Faris (1928)	*H. sacchari*	19.0– 71	.....	52.6	9.4–15.0	.....	12.5	.....	5.5	.....	.....
Drechsler	*H. ocellum*	32.0–103	.....	71.0	9.0–17.0	.....	14.0	3–10	.....	6.7	Fuliginous
Voorhees	*H. ocellum*	28.0– 90	.....	56.4	8.0–14.0	.....	11.1	3– 9	.....	6.5	Light smoky yellow-brown, slightly curved
Matsumoto and Yamamoto	*H. sacchari*	33.0–101	62–75	.....	11.0–16.0	13–15	.....	3–10	6.8	.....	Smoky-gray to grayish-olive or citrine-drab, long elliptical or fusiform
Martin	*H. sacchari*	.....	.....	.....	.....	.....	.....	4– 8	.....	.....	Slightly curved
Mitra (1931)	*H. sacchari*	30.0–112	.....	68.3	11.5–18.5	.....	15.3	3–11	.....	6.9	Yellowish, light olivaceous- to dark smoky-brown with often a tinge of greenness; cylindrical, long elliptical, often slightly curved
Stevenson and Rands	*H. sacchari*	22.0–110	.....	.....	9.0–21.0	.....	.....	3–10	.....	.....	Olive-green to brown, cylindrical, oblong or elliptical, often slightly curved

[a] Obtained by inspection and measurement of drawings by Cobb (5).

eye-spot in Cuba, Florida, etc. Stevenson and Rands (27) accept the conclusions of Mitra (20, 21), and McRae (19) and list *H. ocellum* as a synonym of *H. sacchari*, which is stated to be a highly variable fungus and apparently existing as a number of different strains with saltation common.

The conidiophores of *H. sacchari* from Napier are nonbranched, many septate, with angular inequalities or geniculations marking the insertion of an originally apical spore. The spores, to be described in more detail later, are straight, moderately or markedly curved, long-elliptical, with the greatest diameter near the middle or at one end, gradually tapering to rounded or slightly pointed ends, and many septate; they germinate from one end or from both ends. In color they vary from light to dark brown.

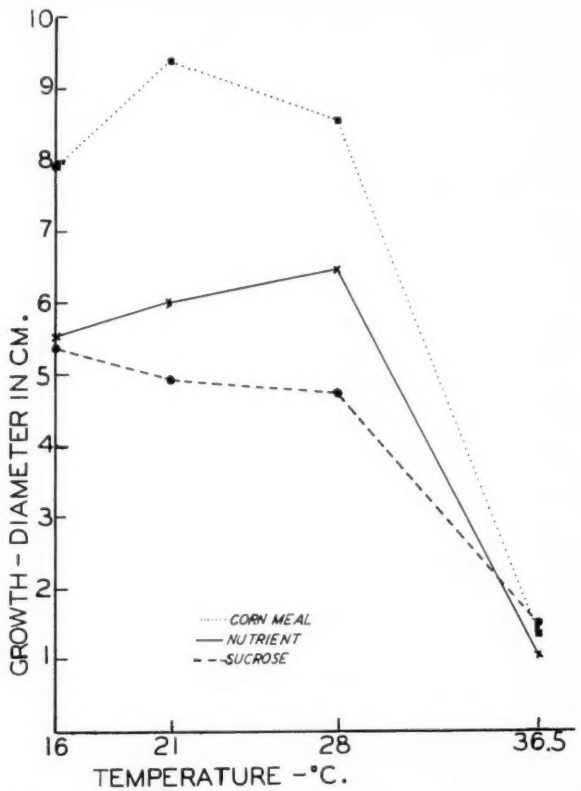

FIG. 2. Effect of temperature on growth of *Helminthosporium sacchari* when cultured on corn-meal agar, standard-nutrient agar and sucrose agar.

Physiology

When the disease appeared on Napier in Hawaii, diseased material was obtained from all of the principal islands of the Hawaiian group, and in most cases from several localities on each island, and the *Helminthosporium* cultured. Ten isolates were thus obtained. A single isolate of *H. sacchari* was cultured from cane diseased with eye-spot, obtained from the grounds

of the Hawaiian Sugar Planters' Association Experiment Station, Honolulu; later, the writer was given 2 additional cultures of *H. sacchari,* isolated from the same locality several years previously, by J. P. Martin, plant pathologist of the sugar station. Napier isolates are hereafter designated in the text as *H. sacchari* (N) and the cane isolate is designated *H. sacchari* (C). The isolates of *H. sacchari* (N) and of *H. sacchari* (C) were studied side by side on various culture media,[3] at different temperatures, to determine growth characters and the formation, size, and color of spores, in alternating light and darkness and in continuous darkness. All media, except Shear's corn-meal agar, were adjusted to pH 7.0.

Effect of Temperature

Growth. The effect of temperature on the growth of *Helminthosporium sacchari* (N) (Fig. 2) was determined, using a single isolate obtained from Kawailoa, Oahu. The fungus was transferred on blocks of corn-meal agar, as nearly equal in size as possible to duplicate plates of standard nutrient, corn-meal, and 1 per cent-sucrose agars, which were placed at 36.5°, 28°, 21° and 16° C. in continuous darkness for 8 days, when the growths obtained were measured. Results, illustrated in figure 2, indicate that *H. sacchari* (N) grows well over the range 16 to 28° C. Somewhat less growth is obtained at the lower than at the higher temperature, except on sucrose where temperature affects the type, as well as the amount, of growth. This point is discussed later. At 36.5° C., growth is slow. The optimum temperature seems to lie between 21° and 28° C. No comparable studies were made with *H. sacchari* (C), but good growth was obtained with all 3 cultures (single isolate) on all media at 21–28° C. Growth was more rapid at the higher figure. Halma and Fawcett (10) have previously shown that *H. sacchari* (C), from Hawaii, has an optimum between 20° and 29° C., with more rapid growth at 29° than at 20° C. Bourne (2) found that 23.5° was optimum for *H. ocellum* from cane, but likewise obtained good growth from 21° to 28° C., while Mitra (20) found that the optimum for *H. sacchari,* in India, was 30° C.

On standard nutrient and on sucrose agars, temperature not only determined the amount of growth of *Helminthosporium sacchari* (N) but also the type of growth. On the former medium, white aerial mycelium was produced at 21° and 28° C., but not at 16° or 36.5° C., and was less abundant at 21° than at 28° C. On sucrose, at 21° and 28° C., the fungus exhibited sectorial growth somewhat similar to that described by Bourne (2) at 28° to 31° C. for *H. ocellum.* At 16° C. growth was somewhat similar to Bourne's picture of the fungus at 23° C.

[3] Potato-dextrose agar— 200 g. potatoes, 10 g. dextrose, 18 g. agar, 1000 cc. water.
Standard nutrient agar—3 g. beef extract, 5 g. peptone, 18 g. agar, 1000 cc. water.
Corn-meal agar— 20 g. corn meal, 18 g. agar, 1000 cc. water (Shear's formula).
Nutrient-dextrose agar—3 g. beef extract, 5 g. peptone, 20 g. dextrose, 18 g. agar, 1000 cc. water.
1 per cent sucrose agar—10 g. sucrose, 18 g. agar, 1000 cc. water.

Spore Formation, Size, and Color

Bourne (2) has reported that *Helminthosporium ocellum* fails to form spores at temperatures up to and including 10.6° C., and at 35° and 38° C. All isolates from Napier and from sugar cane produced spores at 21° and at 28° C. The Kawailoa isolate of *H. sacchari*, which was also studied at 16° and at 36.5° C., produced spores less abundantly at 16° than at 21° or 28°, and did not sporulate at 36.5° C.

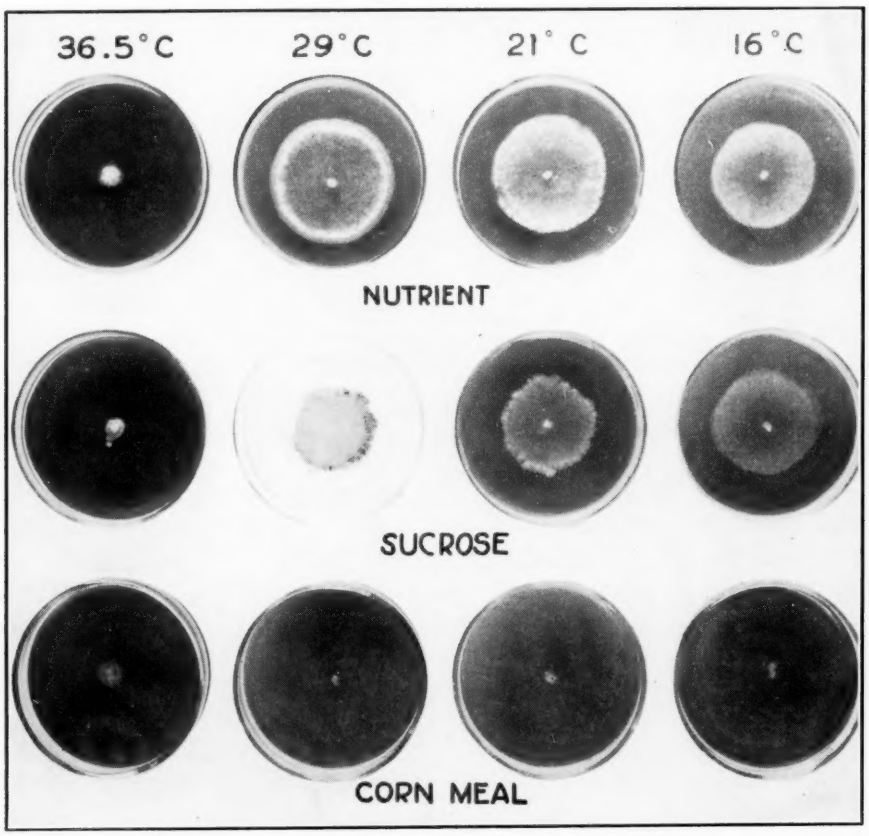

FIG. 3. Effect of temperature and substrate on growth of *Helminthosporium sacchari* (N), isolated from Kawailoa, Oahu; growth in continuous darkness for 8 days.

One hundred fifty spores of each isolate were measured, on each medium, at 21° and at 28° C. Results are summarized in table 2 as ranges and weighted means of length, width, and septation. The color of spores is also recorded. Between different isolates of *Helminthosporium sacchari* (N), considerable variation in spore size, under similar environmental conditions, was found; less variation was found within the single isolate (3 cultures) of *H. sacchari* (C).

The spores of *Helminthosporium sacchari* (N) are longer, narrower and possess more septa when formed at 28° than when produced at 21° C. Con-

TABLE 2.—*Length, width, septation, and color of spores of H. sacchari, from Napier grass and from sugar cane, on four different media at two different temperatures. One hundred fifty spores were measured from each isolate, on each medium, at each temperature*

Plant from which fungus isolated	Number of isolates studied	Medium	Temp. °C.	Length in μ		Width in μ		Number of septa		Color of spores
				Range	Weighted mean	Range	Weighted mean	Range	Weighted mean	
Napier grass	10	Sucrose	28	22.4–128.0	61.6	8.8–16.0	11.6	2–13	6.7	Yellow brown, smoky brown, or dark brown
			21	19.2– 68.8	37.1	9.6–18.4	12.8	2– 8	5.0	ditto
	10	Corn meal	28	25.6–113.6	69.2	8.8–17.6	11.8	3–11	6.6	Yellow brown, smoky brown, or dark brown
			21	25.6– 80.0	47.0	9.6–18.4	13.5	2– 9	5.8	ditto
	2	Nutrient	28	32.0–112.0	64.6	9.6–13.6	11.0	3–10	7.2	Smoky brown to dark brown
	1	Nut.-dex.	28	46.4–110.4	76.2	9.6–14.4	11.7	5–10	7.5	Smoky brown to dark brown
	All media combined		28	22.4–128.0	64.0	8.8–17.6	11.6	2–11	6.7	
			21	19.2– 80.0	41.2	9.6–18.4	13.1	2– 9	5.3	
Sugar cane	1 (3 cultures)	Sucrose	28	27.2– 80.0	55.3	9.6–16.0	12.1	2–10	6.2	Pale brown
			21	35.2– 92.8	67.2	9.6–17.6	13.1	4–10	7.7	Smoky brown to dark brown
		Corn meal	28	27.2– 76.8	55.3	9.6–16.0	11.6	2– 9	5.8	Very pale brown, almost colorless
			21	36.6– 88.0	62.4	9.6–14.4	11.8	3– 8	6.5	Pale brown
		Nutrient	28	25.6– 70.4	47.6	8.8–16.0	12.1	2– 8	3.8	Very pale brown, almost colorless
			21	25.6– 80.8	49.6	9.6–16.8	12.1	2– 7	3.8	Pale brown to very pale brown
		Nut.-dex.	28	25.6– 78.4	45.6	9.6–12.8	11.2	2– 8	4.1	Pale brown
			21	27.2–108.8	68.1	9.6–17.6	13.4	2–10	6.4	Light brown to dark brown
	All media combined		28	25.6– 80.0	49.9	8.8–16.0	11.7	2–10	4.9	
			21	25.6–108.8	62.0	9.6–17.6	12.7	2–10	6.3	

versely, spores of *H. sacchari* (C) are smaller at 28° than they are at 21° C., except on *standard nutrient* agar, where spore size is little affected by a change in temperature. At 28°, the mean spore length of *H. sacchari* (N) corresponds very closely to the mean spore length of *H. sacchari* (C) at 21° C. Mitra (20) has shown that *H. sacchari*, from cane, produces larger spores at 20° than at 30° C., and this is in agreement with local findings for *H. sacchari* (C).

That such differences can be obtained at different temperatures, emphasizes the importance of recording the temperature at which the fungus was grown when spores of *Helminthosporium sacchari* are being measured.

For some isolates of *Helminthosporium sacchari* (N), temperature had little or no effect on spore color, but for other isolates the spores were darker at 21° than at 28° C. At the higher temperature, several isolates produced spores that were lemon-yellow-brown or light yellow-brown with a greenish tint. Spores of *H. sacchari* (C) were consistently darker at 21° than at 28° C. The yellowish tint noted above for spores of *H. sacchari* (N) was absent in spores of *H. sacchari* (C).

Effect of Isolate

Approximately the same type of growth was obtained when the 3 cultures (single isolate) of *Helminthosporium sacchari* (C) were compared on the same substrate at room temperature (27–29° C.). In marked contrast, considerable variability in type of growth was found within the isolates (usually 7 in number but occasionally 8) of *H. sacchari* (N). On certain media the differences were particularly outstanding. Cultures were held in continuous darkness or in alternating light and darkness. Zonate rings were formed with fluctuating light, absent in continuous darkness (Fig. 4).

Growth. There was little or no difference in the growth of *Helminthosporium sacchari* (N) and the growth of *H. sacchari* (C) on potato-dextrose agar. Growth was very rapid, the fungus producing a thick mat of grayish-green, markedly aerial mycelium. Peripheral growth remained devoid of color until a plate was completely covered. *H. sacchari* (C) grew slightly more rapidly and tended to produce a greener mycelium in continuous darkness than in alternating light and darkness. The under side of an old plate culture was purplish-black, but the medium was not pigmented.

On standard nutrient, *Helminthosporium sacchari* (C) formed a light brown, slightly aerial mycelium (Fig. 4). Some isolates of *H. sacchari* (N), for example the Kawailoa isolate, also shown in figure 3, resembled *H. sacchari* (C) but differed by producing white aerial mycelium at the center and periphery of plate cultures. The Kawailoa type of growth also was obtained with isolates from Ulupalakua (Maui), Waimea (Oahu and Hawaii), East Molokai, and Hanalei (Kauai), as is illustrated in figure 5. The isolate from Mokuleia (Oahu) resembled *H. sacchari* (C) but grew more slowly, while the Kona isolate differed from all other Napier isolates by sporulating vigorously to impart to the culture a bluish-black color.

On corn meal, *Helminthosporium sacchari* (C) formed a sparse, non-aerial, rapidly growing, almost colorless mycelium; 6 isolates of *H. sacchari* (N) grew similarly on corn meal, but the isolates from East Molokai and Hanalei in addition produced aerial mycelia.

On nutrient-dextrose, *Helminthosporium sacchari* (C) produced a luxuriant growth of aerial mycelium, at first colorless, later greenish-gray, and finally brownish-black. White tufts of mycelium were interspersed throughout the culture, or more than one type of growth was obtained, evidence that mutations occur within the species. Submerged hyphae were dark in color, but the medium was not pigmented. Crystals were common in old cultures. The growth of *H. sacchari* (N) was variable on this medium, depending on the isolate studied. In general, four types of growth have been found, namely: (a) Luxuriant, grayish, aerial mycelium with occasional tufts of almost pure white hyphae irregularly distributed; obtained with isolates from Waimea (Oahu and Hawaii), East Molokai, and Ulupalakua. (b) Grayish-white aerial mycelium plus abundant, almost pure white aerial mycelium, the latter not necessarily in tufts, the growth very irregular in radial expansion; obtained with Mokuleia isolate. (c) Grayish-white mycelium, evenly overgrown with abundant white mycelium; obtained with Hanalei isolate. (d) Abundantly produced spores giving a bluish-black color to surface of culture with little or no white mycelium visible until several days after surface of a plate is covered, when a few tufts may appear, particularly at the center of the plate; obtained with Kona isolate.

On 1 per cent sucrose agar, in alternate light and darkness, *Helminthosporium sacchari* (C) formed a zonately ringed growth, which was dark greenish-brown by transmitted light and dark-brown with a greenish tint by reflected light. The type of growth resembled that pictured by Bourne (2) for *H. ocellum* at 33° C. In continuous darkness, there was reduction in growth (Fig. 4); the center of a plate culture was light brown with a greenish tint, while peripheral growth was dark green, almost black, and irregular. The older the culture, the more distinct were the colors.

The Kawailoa isolate of *Helminthosporium sacchari* (N) was the only one studied on sucrose in alternate light and darkness. Growth was at first light-brown soon changing to dark-green, with an irregular, slightly dichotomously branched margin (Fig. 4). Zonate ringing was present but was not pronounced. In continuous darkness, growth of this isolate was more rapid (Fig. 4) and the fungus resembled *H. ocellum* at 31° C., as pictured by Bourne (2). Isolates from Waimea (Oahu) and Ulupalakua grew similarly on sucrose, but with other isolates, 3 additional types of growth were obtained, namely: (a) Mycelium light yellow-green with margin of culture slightly darker; obtained with isolates from Waimea (Hawaii) and East Molokai. (b) Same as Kawailoa isolate, but fungus produced peripheral sectors of grayish-brown mycelium, reminiscent of the growth of the Napier fungus on nutrient-dextrose; obtained with Hanalei isolate. (c) Faster

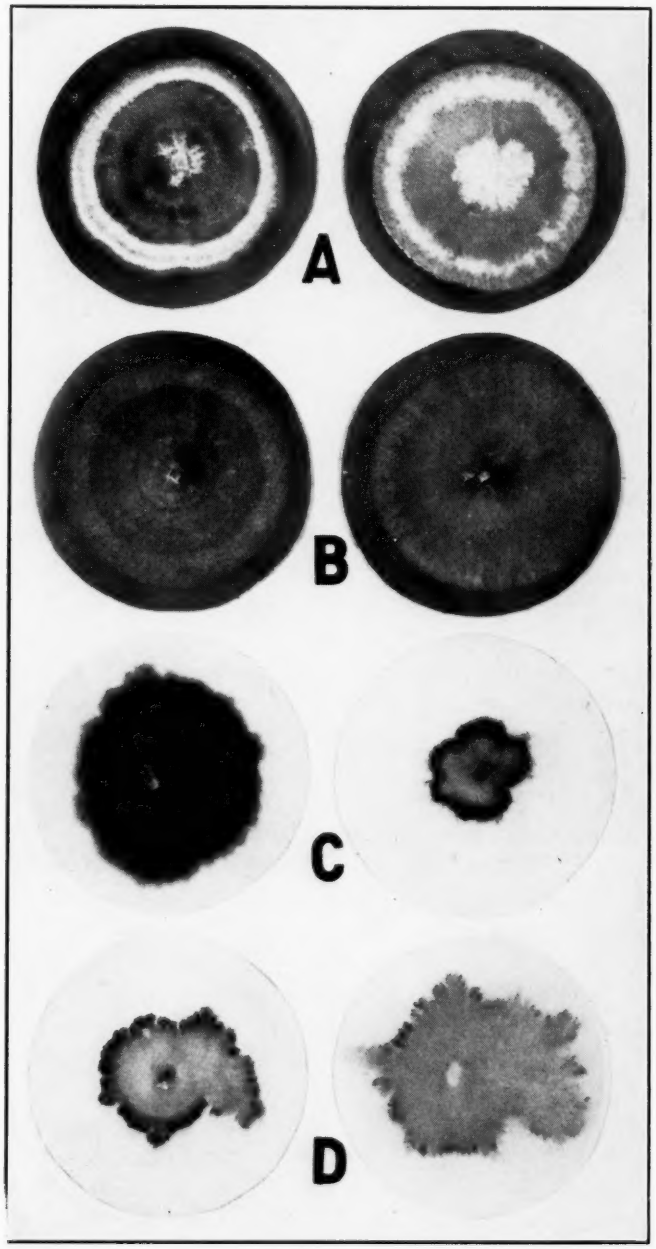

FIG. 4. Effect of substrate and light on growth of *Helminthosporium sacchari* (N), (A and D) and on *H. sacchari* (C), (B and C). A and B on standard nutrient agar; C and D on sucrose agar. Four cultures on left exposed to alternate light and darkness; four on right grown in continuous darkness. Isolate of *H. sacchari* (N) from Kawailoa, Oahu.

growth than obtained with other isolates, darker, with dichotomous branching pronounced; obtained with isolates from Kona and Mokuleia. The last isolate formed some white aerial mycelium (Fig. 5).

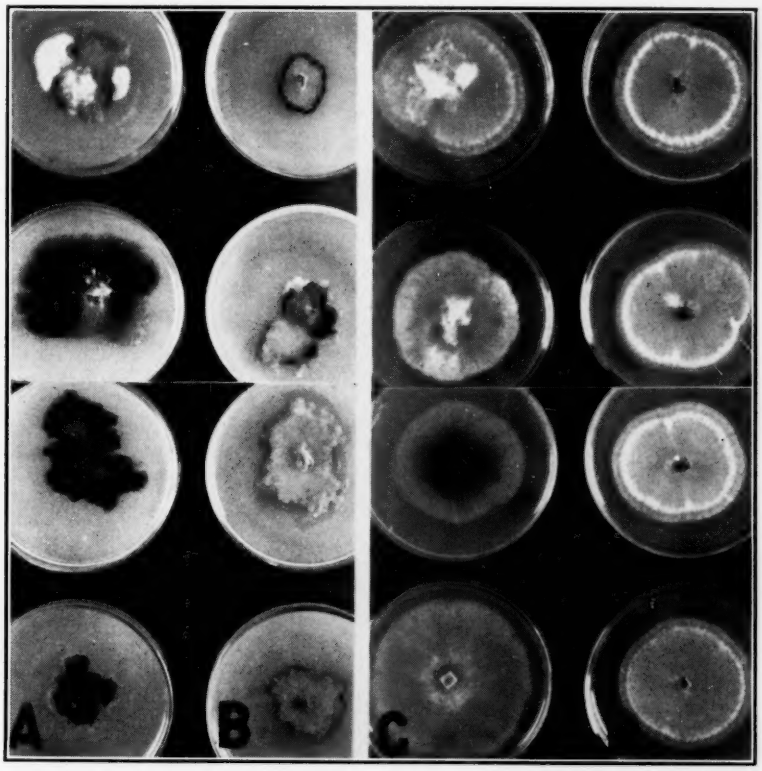

FIG. 5. Cultural variability in 8 isolates of *Helminthosporium sacchari* from Napier grass and cane when grown on sucrose agar (A and B) and standard nutrient agar (C and D). Order in which isolates occur from top to bottom: A and C, Napier from Hanalei, Napier from Mokuleia, Napier from Kona, and cane from Makiki; B and D, Napier from Ulupalakua, Napier from Waimea (Oahu), Napier from Waimea (Hawaii), and Napier from East Molokai.

Substrate had less effect in changing spore size than did temperature. However, spores of *Helminthosporium sacchari* (C) were consistently shorter on nutrient-dextrose than on any other medium. Few to no spores were produced by *H. sacchari* (N or C) on potato-dextrose agar. The Kona and Hanalei isolates were unique in that they sporulated on standard nutrient as well as on corn-meal and on sucrose; the Kona isolate in addition sporulated on nutrient-dextrose, thereby resembling *H. sacchari* (C) more closely than any other isolate of *H. sacchari* (N).

The color of the spores of *H. sacchari* (N) varied from yellowish-brown through smoky-brown to dark-brown, depending on the isolate and the substrate; spores of *H. sacchari* (C) varied from very pale-brown (almost colorless) through smoky-brown to dark-brown.

Effect of Previous Growth Medium

Growth of *Helminthosporium sacchari* (C) and of *H. sacchari* (N)[4] was influenced by the medium from which the inoculum was obtained, particularly when the fungus was grown on 1 per cent sucrose (Fig. 6). If inoculum was taken from standard nutrient, subsequent growth on sucrose was faster than if the inoculum was obtained from sucrose or from corn meal *H. sacchari* (C) produced white tufts of mycelium on sucrose when transferred from nutrient but not when the inoculum was transferred from sucrose or corn meal. These differences were not observed when the fungus was transferred to corn-meal or to nutrient from any other medium.

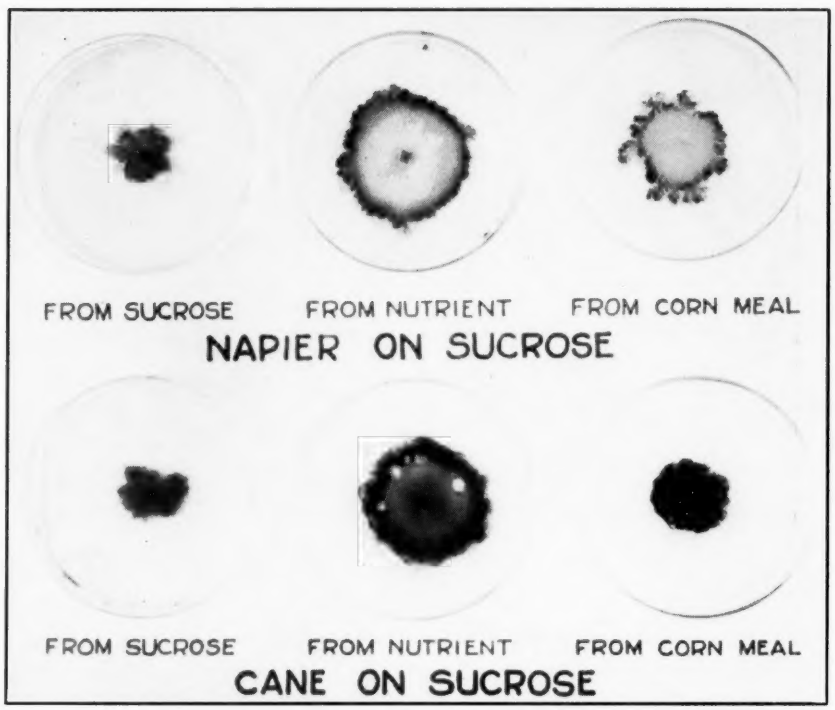

FIG. 6. Effect of previous growth medium on amount and type of growth of *Helminthosporium sacchari* on sucrose agar. Fungus isolated from cane and from Napier (Kawailoa isolate); 8 days' growth at 28° C. in continuous darkness. Note that greatest growth is made when source of inoculum is standard nutrient. Other points of difference are discussed in the text.

As previously mentioned, Mitra (20, 21) and McRae (19) have shown that *H. sacchari* is a highly variable fungus. From the foregoing studies, the writer agrees with these workers, and believes that the differences demonstrated here between *H. sacchari* (N) and *H. sacchari* (C) do not justify a species separation. It is concluded that eye-spot of Napier and eye-spot of sugar cane in Hawaii, and probably elsewhere, are caused by different strains of *H. sacchari*.

[4] Kawailoa isolate only was subjected to this study.

Pathogenicity

The fungus is easily isolated in pure culture from diseased Napier leaf or stem lesions. Spores were atomized on healthy Napier plants which were then placed in a moist chamber for 12 to 18 hours, removed, and transferred to the greenhouse. Symptoms appeared in 2 to 3 days and closely resembled those observed in the field but seldom attained equal severity. From the artificially induced lesions, the fungus was re-isolated and identified in pure culture, thus completing Koch's rules of proof. Voorhees (29) has also demonstrated the pathogenicity of *Helminthosporium sacchari* (*ocellum*) to Napier.

On varieties of sugar cane susceptible to *Helminthosporium sacchari* (C), strains of *H. sacchari* (N) fail to produce typical eye-spot.[5] Small, linear rather than elongate lesions are formed, and runners are absent. The Napier strains are not regarded locally as a menace to sugar-cane production. The writer has demonstrated that cane varieties resistant to *H. sacchari* (C) are not affected by any of the 10 isolates of *H. sacchari* (N).

Helminthosporium sacchari (C), single isolate only, was tested for its pathogenicity to Napier. The plant is susceptible, but lesions are small and not of economic importance. Merker grass, highly resistant to *H. sacchari* (N), is apparently immune from *H. sacchari* (C).

EPIPHYTOLOGY

Unlike eye-spot on sugar cane, which is most severe in Hawaii during the winter months (16), eye-spot on Napier may be severe at any time of the year. Plants suffering from drought or inadequate irrigation appear to be more susceptible and are killed more rapidly than those receiving adequate moisture.

No effect on the severity of eye-spot has been noted from differential fertilization of Napier with N, P, or K; the disease thus differs from eye-spot of cane, which is severe on plants receiving heavy applications of nitrogen (16).

CONTROL

The disease can be controlled in large measure by replacing Napier with Merker grass or selections from reciprocal Napier × Merker crosses (22). This phase of the problem is discussed in detail elsewhere (23).

SUMMARY

A disease of Napier grass (*Pennisetum purpureum* Schum.) in Hawaii, causing eye-spot of the foliage and cankers on the stems, is described. The disease, which first appeared in October 1939, has caused severe losses; entire plantings of Napier have been rendered useless for fodder and many fields have been plowed and replanted with other grasses.

[5] Verbal statement to the writer by C. W. Carpenter, plant pathologist of the Hawaiian Sugar Planters' Association Experiment Station, with whose permission the above is released.

The causal agent is *Helminthosporium sacchari* (van Breda de Haan) Butler. Eye-spot of Napier in Florida has been attributed by Stokes and Ritchey (28) and by Voorhees (29) to *H. ocellum* Faris, also the cause of eye-spot of sugar cane, which Mitra (20, 21) and McRae (19) have shown is a synonym of *H. sacchari*. Evidence presented herein confirms this synonymy.

The physiology of 8 isolates of the fungus from Napier and one isolate (3 cultures) of *H. sacchari* from sugar cane was compared. The Napier fungus has an optimum temperature between 21° and 28° C.; the optimum for the fungus from cane has been variously reported as 20°–29° C., 23.5° C., and 30° C. On certain substrates, at uniform temperature, there was little or no difference in appearance of the fungus isolates from Napier and from sugar cane; on other substrates the cane fungus resembled certain Napier isolates and was distinct from other Napier isolates. Within the isolates from Napier, great variation in type of growth occurred on certain media. Spores formed on 4 different substrates at 2 different temperatures (21° and 28° C.) were measured. The fungus isolated from Napier produced larger spores at 28° than at 21° C.; when isolated from cane the spores were larger at 21° than at 28° C. These differences are not believed to be due to different species of *Helminthosporium*, but to different strains of a single species, *H. sacchari*.

The Napier fungus was isolated, inoculated to healthy plants, the disease reproduced and the fungus recovered in pure culture. Inoculated to sugar cane, Napier isolates have little effect and the same is true of *Helminthosporium sacchari* isolated from cane and inoculated to Napier.

Merker grass, a variety of Napier, and certain reciprocal Napier × Merker crosses are highly resistant. The local disease is controlled by substitution of this resistant material for Napier.

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REACTION OF PEA VARIETIES TO SEPTORIA PISI

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In 1936, while investigating certain virus diseases of peas (*Pisum sativum* L.) in Colorado, the leaf spot (*Septoria pisi* West.) was noted among certain varieties of foreign origin. It was believed that the initial infection originated from the seed. Throughout the growing season, the disease spread naturally to quite a number of other varieties planted in the same plot causing considerable damage to most of them. Since the preliminary evidence indicated that tolerance or resistance might not be common, a study of varietal reaction to the disease was conducted.

Although this disease has been known for many years and has been reported from nearly all sections where peas are grown, it has never caused serious damage to large acreages of either canning or market garden varieties. From its general prevalence in the experimental plantings and from the damage caused, it seemed likely that the disease could produce considerable destruction to commercial plantings if conditions prevailed that were ideal for the spread and development of the causal organism.

Reports have indicated that the disease is an important factor in plantings of Austrian Winter field peas in certain sections of the country. In 1936, Weimer[1] reported this fungus as being the most generally distributed of any of the fungi attacking peas in Georgia. More recently Sprague,[2] reported that *Septoria pisi* in western Oregon is often the sole cause of severe injury.

The purpose of this paper[3] is to record the varietal reaction of a number of pea varieties to this fungus.

METHODS

These studies were conducted from 1936 to 1939 under field conditions in Colorado. The data collected during the first 2 years of the study were in conjunction with experimental plantings principally designed for an investigation of certain virus diseases of peas. Not all of the varieties were tested every year. In certain years they were replicated; in others they were not. With one exception each variety was tested at least 3 times in 2 years and a few of the varieties in each of 4 years. The number of plants of each variety tested in any one year ranged from 20 to 70 with an average of approximately 35–40 plants.

When the plants were about 4–6 in. tall they were sprayed with a spore suspension of the organism, by using a 2-gallon pressure spray tank.

[1] Weimer, J. L. Diseases of Austrian Winter peas. U. S. Dept. Agr., Bur. Pl. Ind., Plant Disease Rptr. 20: 210–212. 1936.

[2] Sprague, R. Notes on Septoria scald on vetch and peas in Oregon. Phytopath. 30: 541–542. 1940.

[3] The writer expresses his appreciation to E. C. Tatman and V. R. Boswell for advice and assistance in the statistical analysis of the data.

Infected pea straw was used as the source of inoculum. The straw was immersed in water for a short period to liberate the spores. The spore suspension was then strained through cheesecloth and used to spray the young pea seedlings. The inoculation was performed in the evening in order to retard as much as possible the evaporation of the moisture from the inoculated plants. A second inoculation was made about 2 weeks following the first in order to be assured of a uniform infection. About 3 weeks after the first inoculation, wide-spread infection was noted on the lower leaves of many varieties. Frequent showers and, in certain years, an occasional light hail were in a large measure responsible for the amount and rate of infection.

Infection ratings were made 3 times throughout the season. At the end of approximately 10 weeks, plants showing mild infection were given a rating of 1 to 2, moderate infection 2 to 3, severe infection 3 to 4, and very severe infection 4 to 4+. The latter included plants that had died as a result of the disease.

The data were analyzed according to Fishers' method for the analysis of variance. Owing to inconsistencies in the experimental design, it was impossible to set up a single analysis which would cover the results of all the tests. Hence the data were analyzed as several smaller tables, each covering certain varieties tested in 2 or more years in single or replicate tests.

SYMPTOMS

The initial symptoms were noted as yellowish-green indefinite areas on the leaves, which gradually darkened and enlarged until the entire leaflet was involved. Later the petioles and stipules became infected, followed by the nodes which became shrunken and sometimes girdled. In the lesions, pycnidia developed within a few weeks following infection. When infec-

TABLE 1.—*Summary of variance analyses of the reaction of 131 pea varieties and strains to Septoria pisi*

Number of varieties and strains compared	Years compared	Blocks	Differences due to[a]	
			Years	Varieties
4	1936, 1937, 1938, 1939	1	−	−
10	1936, 1937, 1939	1	−	++
5	1937, 1938, 1939	1	−	−
8	1936, 1938, 1939	1	−	+
5	1936, 1938, 1939	2	−	++
71	1936, 1937, 1938	1	−	++
6	1936, 1939	2	−	++
9	1936, 1939	1	−	++
13	1937, 1939	1	−	+
42	1938, 1939	2	+	++
81	1936, 1937	1	−	++
97	1937, 1938	1	−	++
53	1936, 1938	2	−	++
78	1936, 1938	1	−	++

[a] Minus sign (−) represents non-significant; (+) significant; (++) highly significant.

TABLE 2.—*Reaction of peas to Septoria pisi*

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
I. Canning Varieties				
Alaska 2B	E	2	3	4.0 ± .27
Alaska, Asgrow	E	3	5	3.9 ± .32
Alaska Large Podded	E	3	4	3.9 ± .36
Alaska, Maryland	E	3	4	4.2 ± .36
Asgrow Canner King	M	2	4	2.7 ± .19
Asgrow Early Harvest	E	2	3	3.8 ± .27
Asgrow Pride	M	2	3	2.3 ± .27
Asgrow Triumph	M	2	3	3.3 ± .27
Bruce	L	3	5	2.8 ± .32
Canners Delight	L	2	4	1.4 ± .19
Canners Gem	M	3	5	2.2 ± .32
Canners Gem	M	2	3	2.7 ± .27
Chief	M	2	3	2.8 ± .27
Climax	M	2	3	2.3 ± .27
Green Admiral	L	2	3	3.0 ± .27
Green Admiral	L	4	6	2.8 ± .57
Green Giant	L	3	5	2.7 ± .32
Horal	L	3	4	2.8 ± .41
Horsford	L	3	5	2.2 ± .32
No. 58	L	2	3	2.3 ± .27
Major	L	2	4	3.0 ± .19
Market Surprise	E	2	3	4.0 ± .27
Meteor	E	3	5	3.9 ± .32
Perfectah	L	3	5	2.6 ± .32
Perfection	L	3	5	1.1 ± .18
Perfection	L	3	4	2.0 ± .36
Perfection	L	3	5	1.8 ± .32
Perfection, Early	M	2	3	1.7 ± .27
Perfection, Wisconsin	L	3	4	2.2 ± .36
Perfection, Wisconsin	L	3	5	1.7 ± .32
Premium Gem	E	3	5	3.4 ± .32
Premium Gem	E	2	3	2.5 ± .27
Prince of Wales	L	3	4	2.7 ± .36
Profusion	L	3	5	2.9 ± .32
Rice No. 13	L	4	6	4.1 ± .33
Rogers Ace	E	2	4	3.1 ± .19
Rogers Delicious	L	2	4	1.6 ± .19
Rogers Famous	L	2	4	1.9 ± .19
Rogers Gem	M	2	3	2.0 ± .27
Rogers Kay	L	2	4	3.6 ± .19
Senator	L	3	5	2.4 ± .32
Senator	L	2	3	3.0 ± .27
Short Admiral	L	2	3	2.5 ± .27
Stella	E	3	3	4.0 ± .36
Surprise	E	3	5	4.0 ± .32
Winner	E	3	5	3.7 ± .32
Wisconsin Early Sweet	E	3	5	4.0 ± .32
Yellow Admiral	L	3	5	2.6 ± .32
Yorkshire Hero	L	2	4	2.1 ± .19
II. Market Garden Varieties				
Admiral Beatty	L	2	4	3.4 ± .19
Alderman	L	3	5	3.0 ± .36
Alderman	L	3	4	3.0 ± .32
American Wonder	E	3	5	3.6 ± .32
Asgrow No. 40	L	3	5	3.0 ± .32
Banqueteer	E	3	4	3.9 ± .36
Bliss Everbearing	L	2	4	2.5 ± .19

TABLE 2.—(*Continued*)

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
II. Market Garden Varieties—(*Continued*)				
Blue Bantam	E	3	6	3.0 ± .26
Bountiful	E	3	4	3.8 ± .36
British Lion	E	3	4	3.9 ± .36
Carter Eight Weeks	E	3	5	3.8 ± .32
Charles 1st	M	2	3	2.5 ± .36
Confidence	M	2	4	3.5 ± .19
Dark G.O.P.	L	3	3	2.2 ± .40
Duplex	L	3	4	3.3 ± .36
Dwarf Alderman	L	2	3	2.5 ± .27
Dwarf Champion	L	2	3	2.3 ± .27
Dwarf Telephone	L	3	5	2.5 ± .32
Dwarf Telephone	L	3	4	2.7 ± .36
Early Eight Weeks	E	2	4	3.9 ± .22
Early Gilbo	L	3	4	3.0 ± .36
Everbearing	L	3	5	2.4 ± .37
Glacier	E	2	4	3.8 ± .19
Gradus	E	3	5	3.6 ± .32
Gradus	E	2	3	3.0 ± .27
Horsford	L	3	5	2.2 ± .28
Hundredfold	M	3	5	3.5 ± .32
Hundredfold Improved	M	2	2	3.0 ± .38
Hurst Monarch	M	2	4	3.9 ± .19
Kelvendon Wonder	E	3	5	3.7 ± .24
Laxton Progress	E	3	5	3.9 ± .32
Laxton Progress	E	2	3	4.1 ± .27
Laxton Progress	E	3	5	3.8 ± .32
Laxton Superb	E	3	6	3.6 ± .26
Laxtonian	E	2	4	3.0 ± .19
Lincoln	L	3	5	2.2 ± .32
Little Marvel	E	3	5	3.4 ± .32
Lord Chancellor	M	3	3	2.2 ± .36
Nott Excelsior	E	3	5	3.5 ± .32
Onward	L	3	5	2.1 ± .32
Onward	L	2	4	2.9 ± .19
Pedigree Extra Early	E	3	5	4.1 ± .32
Peter Pan	M	2	4	2.7 ± .19
Pilot Improved	E	3	4	4.2 ± .36
Pioneer	E	3	5	3.3 ± .32
Potlatch	L	3	4	2.2 ± .32
President Wilson	M	4	7	3.6 ± .31
Pride of Market	L	3	5	2.1 ± .28
Prolific Early Market	E	2	3	4.0 ± .27
Quite Content	M	3	5	3.2 ± .32
Rogers Giant Podded Hamper	L	2	4	2.9 ± .19
Rogers No. 93	L	3	4	2.5 ± .32
Rogers No. 95	L	2	4	1.9 ± .19
Sharp Miracle	E	2	3	3.7 ± .27
S.S. Pioneer	M	3	4	3.1 ± .31
Stratagem	L	3	4	2.2 ± .43
Stratagem	L	3	5	2.6 ± .32
Sutton Excelsior	M	3	5	3.3 ± .32
Sutton Foremost	E	2	4	4.1 ± .31
Teton	E	2	4	4.1 ± .19
Thomas Laxton	E	3	5	4.0 ± .32
Tom Thumb	L	2	4	2.4 ± .19
World Record	E	2	3	4.2 ± .27
Zwaan Paramount	L	3	4	2.9 ± .31

TABLE 2.—(*Concluded*)

Variety	Time of maturity[a]	No. years tested	Number of tests	Disease index mean and S.E.
III. Edible Podded Varieties				
Dwarf Gray Sugar	M	3	5	2.7 ± .32
Dwarf Gray Sugar	M	3	5	3.1 ± .32
Dwarf White Sugar	M	2	3	2.5 ± .27
Mammoth Melting Sugar	L	3	5	2.7 ± .32
Mammoth Pod Early	E	2	3	4.2 ± .27
Moerheim Giant	L	2	3	3.2 ± .31
Paramount Sugar	M	3	5	3.0 ± .32
IV. Field Varieties				
Austrian Winter	L	3	5	2.5 ± .32
Black Eye Marrowfat	L	3	4	1.6 ± .36
Blue Prussian	L	4	7	1.8 ± .43
Capucijner	L	2	3	2.8 ± .27
Harrison Glory	L	3	4	2.4 ± .36
Harrison Glory	L	2	3	2.5 ± .27
Harrison Glory	L	3	5	3.0 ± .32
Imported (from China)	L	2	4	2.9 ± .19
Imported (from Puerto Rico)	L	2	4	1.1 ± .19
Kootenay	L	3	4	3.2 ± .31
Maple	L	3	5	2.2 ± .32
Swedish Yellow	L	3	4	3.1 ± .36
White Canada	L	3	5	3.1 ± .32
White Eye Marrowfat	L	3	5	2.4 ± .37

[a] E, represents early; M, medium; L, late.

tion took place early, young plants of very susceptible varieties died before producing pods. If the infection was not so serious or occurred when the plants were more mature, a small seed crop was produced.

EXPERIMENTAL RESULTS

The variance analyses showed that, in general, there was no significant difference due to years in which the data were collected, except in one instance (Table 1). There were, however, significant differences due to varieties in all except two tests, which were made up of 4 and 5 varieties, respectively. A summary of these analyses is recorded in table 1.

The canning varieties with their respective mean reaction to the disease are listed in table 2, which shows that only a few varieties exhibited any appreciable degree of resistance. Of the varieties tested, 7 were mildly, 20 moderately, and 22 severely infected. Most of the varieties showing mild infection were of the Perfection type. In addition to these, Canners Delight, Rogers Delicious, and Rogers Famous were also quite tolerant.

None of the market garden varieties exhibited as high a degree of tolerance as the most tolerant canning varieties (Table 2). Of the 64 market garden varieties tested, only one, Rogers No. 95, showed mild infection, 24 were moderately infected and 39 exhibited severe and very severe infection.

None of the edible podded varieties (Table 2) was highly tolerant, but only one, Mammoth Pod Early, was extremely susceptible.

Of the field varieties tested (Table 2) three were mildly infected, seven moderately, and four severely. An import from Puerto Rico exhibited the greatest resistance of any of the varieties tested.

DISCUSSION

It is evident that among the 134 varieties and strains of peas tested, only 2 showed promise from the standpoint of high tolerance to *Septoria pisi*, namely one strain of Perfection and an unnamed import from Puerto Rico. The latter is a very late maturing variety of poor type, whereas the former belongs to a group of important canning varieties. It is possible that either of these strains would be suitable for parental material if hybridization studies for disease resistance were conducted.

There seems to be a relationship between earliness of maturity and extreme susceptibility and also between moderate tolerance and later maturity. Among the 42 early varieties tested in all of the classes, 41 were either severely or very severely infected and one was moderately infected. Table 3

TABLE 3.—*Differences in average mean reaction to Septoria pisi between early, medium and late maturing pea varieties*

Type of pea	Mean disease index for number of varieties of the season of maturity shown							
	Early		Medium		Late		Total	
	No.	Index	No.	Index	No.	Index	No.	Index
Canning	14	3.7	9	2.4	26	2.5	49	2.9
Market Garden	27	3.7	11	3.1	26	2.6	64	3.1
Edible Podded	1	4.2	4	2.8	2	2.9	7	3.3
Field	.....	.....	.....	.....	14	2.4	14	2.4
Total	42	3.9	24	2.8	68	2.6	134	2.9

shows that the group mean reaction of these varieties was 3.9. Among the 24 medium maturing varieties, one was mildly infected, 12 moderately, and 11 severely, and the group mean reaction was 2.8 (Table 3). Of the 68 late varieties, 9 were mildly infected, 42 moderately, 16 severely, and 1 very severely. The mean reaction of this group was 2.6 (Table 3). From this it appears that there is no significant difference between the mean reactions of the medium and the late maturing groups of varieties, but that the difference between the early maturing and the other two groups appears to be significant.

SUMMARY

One hundred and thirty-four strains and varieties of peas were inoculated with *Septoria pisi* under field conditions from 1936 to 1939, in order to determine their relative resistance and susceptibility. Only two varieties

exhibited a very high degree of tolerance. The canning varieties of the Perfection type were in general the most tolerant group. In general the earlier varieties were the more susceptible ones and the later varieties the more tolerant ones.

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ANGULAR LEAF SPOT OF MUSCADINES, CAUSED BY MYCOSPHAERELLA ANGULATA N. SP.[1]

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INTRODUCTION

Muscadine grapes (*Vitis rotundifolia* L.) have not been the subject of careful pathological investigations in the past, hence they are generally regarded as being relatively disease-free.

Studies by the writer, extending over the past 3 years, have brought to light several destructive foliage diseases and berry rots that either have escaped the attention of pathologists or have been assumed to be the same diseases, generally well known on bunch grapes.

Following is a report on the symptomatology and etiology of one of the foliage diseases caused by a pathogen known heretofore as *Cercospora brachypus* Ell. and Ev. (1). Because of the characteristic shape of the lesions on most varieties of muscadines, angular leaf spot of muscadines seems an appropriately descriptive name for the disease.

SUSCEPTS AND RANGE

Field observations and inoculation experiments under controlled conditions indicate that all the common varieties of muscadine grapes are susceptible to angular leaf spot, although not necessarily to the same degree. In general, the older varieties, such as Scuppernong, Flowers, and Thomas, apparently suffer less than the more recent introductions of better quality, as Hunt, Yuga, Creek, Stuckey, Howard, etc. Likewise, field observations and inoculation trials have given no indication that several common varieties of bunch grapes, such as Concord, Delaware, etc., are susceptible to muscadine angular leaf spot. In certain large vineyards in the vicinity of Experiment, Georgia, as well as in several home plantings of a few vines, in which muscadine and bunch grapes have been growing side by side for years, muscadine angular leaf spot could be found only on the muscadine varieties.

Since there has been no literature published on this disease, other than a brief description of the imperfect stage of the pathogen (1, 5), one can do no more than guess as to its range. It might be assumed, however, that the disease is coextensive with muscadine culture. It is certainly the dominant leaf spot on muscadines in the vicinity of Experiment, Georgia; and, since the type material was collected in Alabama, it might safely be assumed that it is present throughout the southeastern States.

SYMPTOMATOLOGY

The earliest evidence of infection appears as small chlorotic areas, most noticeable on the upper leaf surface. Following this, the individual lesions

[1] Paper No. 83, Journal Series, Georgia Agricultural Experiment Station.

develop fairly rapidly and soon a small, dark brown to black, necrotic area appears in the center of each of them (Fig. 1, A, B, C). Continued development results in the formation of angular to irregular lesions, discrete, for the

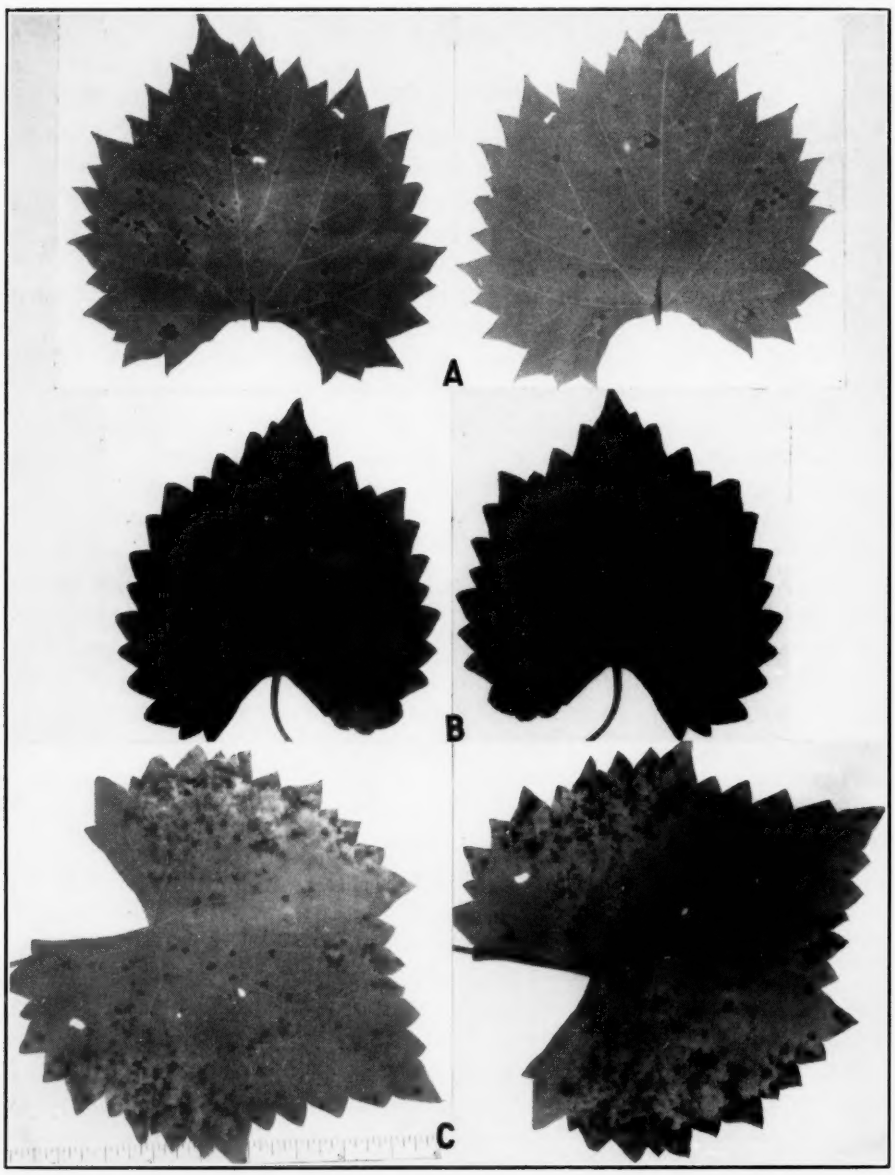

FIG. 1. Photographs showing the symptoms of angular leaf spot of muscadines as they appear on the upper (left) and lower (right) leaf surfaces of several varieties of muscadines. A. on the Hunt; B. on the Yuga; C. on an unnamed male, older lesions. A single lesion of muscadine black rot is present on the Hunt (lower left and right side, respectively). Note the presence of halos on the young lesions. Since the photographs were taken with transmitted light, the halos tend to be evident on the lower surfaces as well as on the upper. $\times \frac{1}{2}$.

most part, and tending to be confined to the areolae of the leaf. At first, distinct halos are present, but these tend to disappear with age. On certain varieties—Hunt and Scuppernong—the lesions tend to become more circular (Fig. 1, A), but, when viewed by transmitted light, the angular outline of the primary lesion is readily apparent. Some coalescence of older lesions occurs on most varieties, but it is usually incomplete, so that most of the original lesion is distinct. At maturity the lesions vary in diameter from a few millimeters to several centimeters.

On the lower surface the halos are indistinct to absent from all lesions. The angular necrotic areas are dark brown to black and appear papillate under magnification, due to the presence of numerous small conidiophore tufts. These tufts are numerous on the upper leaf surface only after the lesions are old, but may appear sparsely at other times. In seasons of high humidity, the conidiophores are produced amphigenously on the older lesions, and are light olive-gray to dark olive-gray. The secondary infection cycles continue to operate throughout the season, but the disease becomes epiphytotic rather late, so that the principal effect is that of premature defoliation.

During relatively normal seasons, conidia are not abundant on field material, but, when leaves containing mature lesions are enclosed over night in moist chambers, abundant conidia are produced on both surfaces of the lesions. Under these conditions of high humidity, the conidiphores tend to become flaccid and these, in conjunction with other hyphae growing out from the lesions, present a byssoid appearance, particularly on the lower leaf surface.

ETIOLOGY

A fungus causing a spotting of the leaves of muscadines was collected near Tuskegee, Alabama, by G. W. Carver and sent to Ellis, who, in 1902, described the fungus as *Cercospora brachypus* Ell. and Ev. Since, to the writer's knowledge, this is the only species of *Cercospora* that has been described on muscadines, he was reasonably certain that his collections represented Ellis' fungus. However, since the original description is so brief and no information has been published subsequently, field collections were sent to several taxonomists for comparison with the type material.[2] The general consensus is that the present material is identical with *Cercospora brachypus* Ell. and Ev., although Ellis' material shows larger and more circular lesions than did the material submitted by the writer. Since all other details, however, are in agreement, and particularly since the writer has since found considerable variation in the size and conformation of lesions on various varieties of muscadines, it is felt that the above mentioned discrepancy is not taxonomically significant.

A study of the life cycle of the pathogen has shown that in addition to the imperfect stage, the fungus develops spermogonia and perithecia in over-

[2] Acknowledgment is made to Charles Chupp, J. A. Stevenson, and F. J. Seaver for their expressions of opinion as to the identity of the pathogen.

wintering leaves, and, as the perfect stage is an undescribed *Mycosphaerella*, it will hereafter be referred to as **Mycosphaerella angulata** n. sp.

DEVELOPMENTAL MORPHOLOGY

Evidence secured from numerous controlled inoculations using both conidia and ascospores indicates that infection may occur through either leaf surface, and that penetration is both direct and through the stomata. The infection hypha is first intercellular, but, as the host cells die rapidly in advance of the advancing mycelium, an intracellular relationship is established early. No evidence of haustoria has been found. The conidiophore tufts first develop on the lower leaf surface, as stated above, but often become amphigenous with age and high humidity. They originate from subcuticular or subepidermal hyphae and usually begin to produce conidia shortly after they emerge from the leaf surface, long before they assume the characteristic complexity of structure commonly assigned to them. As they grow older, the basal mass is built up into a definite stroma with short emergent fertile hyphae (Fig. 2, A, B).

With age and subsequent cyclic development, spermogonia and perithecia often develop in these old conidiophore bases. Undoubtedly the unsuspected presence of early stages of these reproductive bodies influenced Ellis to describe the conidiophore bases as "sphaeriform." The basal portions of the conidiophores quickly become pigmented, while the aerial portions range in color from almost hyaline to dark olive-gray. Under fairly dry conditions, the fascicles resemble short tufts, the conidia are not abundant, while, under conditions of fairly high humidity, the conidiophores become byssoid in appearance. In the latter case, conidia are abjointed from hyphae that resemble vegetative mycelium, except for the presence of geniculations (Fig. 2, D).

The conidia are hyaline, or nearly so, slender, curved, more or less acute at each end, 1–5 septate, with usually two oil droplets in each cell and measure 16.8–112 × 2.24–3.5 μ. The length and number of septations are influenced by relative humidity. Under average field conditions the conidia measure rather uniformly 47.6–72 × 2.8–3.5 μ. It would thus appear that measurements based on width are more reliable taxonomically than are those based on length.

Conidia germinate uniformly within 3 to 8 hours on a 2 per cent tap agar, when conditions of temperature and oxygen balance are favorable. The germ tubes emerge from the terminal cell at either end or both ends of the spore, and often from other cells, as well.

As stated above the spermogonia often develop within the stromata comprising the conidiophore bases. They may also develop concurrently from more recent infections that apparently never have produced conidia. Spermogonia, like perithecia, have not heretofore been reported for the parasite under consideration, but since the writer and others (3, 4, 2) have repeatedly reported on the development of these structures in the course of studies on

other fungi, no great effort has been made toward a critical study of the spermogonia of *Mycosphaerella angulata*. They are initiated in late September and early October; and, while a majority of them mature in October and early November, various developmental stages may be found as late as early spring on the overwintering leaves. They originate in either leaf sur-

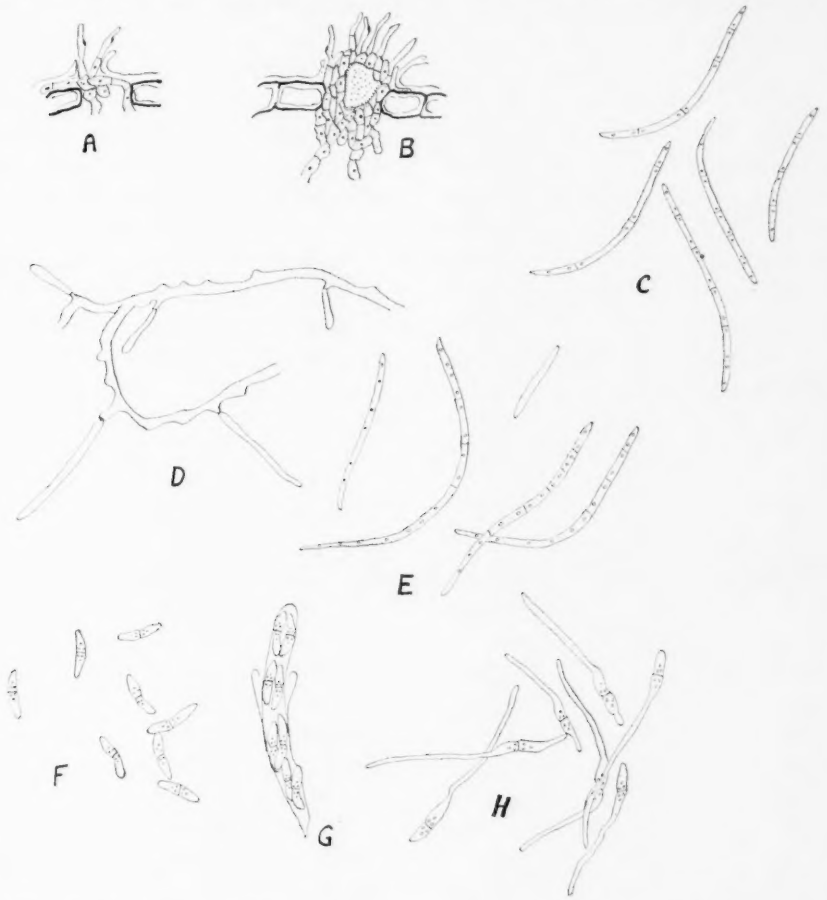

FIG. 2. Spore forms of *Mycosphaerella angulata*. A. Young conidial stroma before a sphaeriform base is evident. B. An older conidiophore fascicle, showing a well-developed basal stroma in which a spermogonium is developing. C–E. Conidia: C, from ascospore culture on 2 per cent potato-dextrose agar; D, from field material left in a moist chamber overnight (note the byssoid conidiophores); E, from field material. F–H. Ascospores: F, appearance when first discharged on agar; G, a mature ascus showing its bitunicate nature; H, germinating ascospores. (All sketches made with the aid of a camera lucida. ×750.)

face, but are perhaps more often epiphyllous. The details of development are almost identical with those described in other studies (3, 4), and, at maturity, the spermatia occur in groups of 3 or 4 and are liberated through sterigma-like processes from the spermatiferous cells. Mature spermatia are rod-shape, uninucleate, and measure 2–4 × 0.5–0.7 μ.

Perithecial development is initiated concurrently with the spermogonia and in similar positions in the leaf tissue. Those originating outside conidiophore bases frequently begin their development from subcuticular hyphae. Soon one to several deep-staining archicarps, each with an elongate trichogyne and an uninucleate basal cell, become evident. At this stage of development perithecial fundaments are readily distinguishable from young spermogonia.

Due to certain peculiarities in time and mode of development, which have increased certain technical difficulties, a full report on the cytological development of the perithecia will be deferred until a later date. However, it can be stated at this time that the evidence is strong that the spermatia function as male sexual elements and that spermatization is essential to the initiation and maturation of ascospores.

When the perithecia are mature, the asci discharge their spores readily on slight drying. The asci do not mature at one and the same time within a given perithecium, so that the source of primary inoculum is present for some time in the field during the spring. Climatic conditions, particularly showers and subsequent drying, greatly influence the span of the primary cycle.

DEVELOPMENT IN CULTURE

Single and multiple conidial isolates were obtained by streaking spore suspensions on the surface of hard tap agar, while ascospore isolates were obtained by allowing the spores to discharge upward and stick to the surface of inverted agar plates. By the latter method, single or multiple spore isolations could be had at will, free from contamination, by regulating the distance between the perithecia and the surface of the agar plates. Mature spores were measured both in crushed mounts and immediately after their discharge into the agar plates. When the conditions of elevation and humidity are favorable, one can cause a majority of the mature asci within a perithecium to discharge their spores into the agar within 10 to 15 minutes after setting the spore traps. Under these conditions the ascospores are hyaline, bicellular, straight to slightly curved, guttulate, and measure 14–19 × 2.8–5.6 μ (Fig. 2, F, G).

For purposes of comparison, only modified potato-dextrose and malt agars were used. Initial growth and pigmentation were the same on both agars, but isolates from both ascospores and conidia continued to sporulate longer on the malt agar.

Both types of spores germinated readily in from 3 to 8 hours and produced visible growth in about 3 days. In all measurable respects, cultures from conidia and ascospores are identical. When first visible, the mycelium is pale gray to almost white, but after the colonies are several millimeters in diameter, the color ranges from light olive-gray to dark olive-gray; essentially all the color being in the vicinity of the substrate. Isolates from both sources sporulate abundantly within three to five days, but sub-cultures

from both types of cultures tend to remain sterile. In this work no great care was taken to sub-culture spores, only, and it may be that such cultures would continue to sporulate whereas sub-cultured mycelium does not. Conidia produced on ascospore isolates are identical with those produced on conidial isolates, and both in turn are identical with conidia produced on field material under similar conditions of temperature and moisture (Fig. 2, C, D, E). Unlike isolates of other Mycosphaerellas reported earlier (2), cultures of *M. angulata* do not undergo much color change even after months on agar, and never become generally stromatic throughout. Very few spermogonia have been found in culture on the media used, and no evidence of perithecia has been seen.

Both conidia and ascospores were used in the inoculation trials. Conidial inoculum was obtained direct from field material, from conidial isolates, and from ascospore isolates; whereas ascospores were obtained from field material, as described above. In all cases resultant infections were identical. The first recognizable infections appeared in the greenhouse 10 days to 2 weeks following inoculation, while the checks remained free from infection in all cases. The above evidence, taken as a body, is convincing proof that the 3 spore forms that have been considered are, genetically, but phases in the reproductive cycle of one and the same organism.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the bicellular, hyaline spores are clearly characteristic of the genus *Mycosphaerella* Johans. Since no other *Mycosphaerella* has heretofore been reported on the foliage of *Vitis rotundifolia* L., and since there is no evidence to indicate that any of the varieties of bunch grape are susceptible to the pathogen under consideration, there can be little doubt that this is an undescribed species. It is, therefore, designated **Mycosphaerella angulata** n. sp.[3] with the following diagnosis:

Mycosphaerella angulata n. sp.

Syn: *Cercospora brachypus* Ell. and Ev. (Jour. Mycol. 8: 71, 1902).

Perithecia scattered, mostly in lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, beaked prior to maturity, 40–90 × 40–60 μ, black, ostiolum papillate when mature; asci cylindrical club-shape, short stipitate, fasciculate, 36.4–42 × 8.4–14 μ, aparaphysate, bitunicate, eight-spore; spores uniseriate to imperfectly biseriate in the ascus, bicellular, straight to slightly curved, hyaline, guttulate, 14–19.6 × 2.8–5.6 μ (average 16.8 × 4 μ).

Hab. In overwintered lesions produced by the conidial stage on leaves of *Vitis rotundifolia* L., Experiment, Ga., maturing during spring.

Spermogonia: Scattered in and along margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous but perhaps more often epiphyllous, embedded

[3] Having reference to the shape of the lesions it produces on the foliage of most varieties of *Vitis rotundifolia* L.

in leaf tissue but later erumpent, ostiolate, 30–60 × 30–50 μ; spermatia small, rod-shape, hyaline, 2–4 × 0.5–0.7 μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

Hab. On recently fallen leaves, maturing throughout period from October through February.

Conidial stage: Lesions mostly angular, often confluent, varying in size, 1 mm. to several cm., dark-brown to almost black, surrounded on upper surface by a distinct halo when young, confined to leaves; conidiophores mostly hypophyllous, becoming amphigenous with age, base becoming stromatic, fasciculate to lax, geniculate, pigmented at base, continuous to one to several septae, mostly short; conidia colorless or nearly so, very slender, cylindrical, acute at each end, curved, 16.8–112 × 2.24–3.5 μ (mostly 47.6–72 × 2.8–3.5 μ), 1–5 septate, guttulate, length and septation influenced by humidity.

Hab. Parasitic on leaves of *Vitis rotundifolia* L., causing angular leaf spots and contributing to premature defoliation.

Peritheciis sparsis, plerumque in maculis, amphigenis, semi-immersis, punctiformibus, ovatis vel globatis, 40–90 × 40–60 μ, rostratis cum immaturis, nigris, ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, fasciculatis, aparaphysatis, bitunicatis, octosporis, 36.4–42 × 8.4–14 μ; sporiidis uniseriatis vel biseriatis, bicellularibus, vix curvatis, hyalinis, guttulatis, 14–19 × 2.8–5.6 μ, plerumque 16.8 × 4 μ.

Hab. in foliis dejectis *Vitis rotundifoliae*, Experiment, Ga.

Spermogoniis autumno efformatis, sparsis, plerumque in maculis et marginatis, ovatis vel globatis, nigris, amphigenis plerumque epiphyllis, innato- erumpentibus, punctiformibus, 30–60 × 30–50 μ; spermatiis bacillaribus, hyalinis, 2–4 × 0.5–0.7 μ.

Hab. in foliis dejectis *Vitis rotundifoliae*.

Statu conidico in maculis angularibus v. irregularibus, confluentibus, rubroferrugineis v. nigris, pallide marginatis, foliis, efformato; hyphis fertilibus plerumque hypophyllis, rare amphigenis, a stromate orientibus, fasciculatis v. byssoideis, geniculatis, hyalinis v. dilute olivaceis-griseis, continuis v. pleuriseptatis, brevisme; conidiis hyalinis, rare sub-hyalinis, angustio-cylindraceis, utrinque acutis, curvulis, 16.8–112 × 2.24–3.5 μ (plerumque 47.6–72 × 2.8–3.5 μ) 1–5 septatis, guttulatis.

Hab. in foliis vivis *Vitis rotundifoliae*.

For the convenience of plant pathologists and mycologists, material has been deposited in the following herbaria: Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., Farlow Herbarium; Plant Pathology Department, Cornell University, Ithaca, N. Y., and Herbarium of the Georgia Experiment Station, Experiment, Ga.

CONTROL

Results obtained from a single season's work indicate that angular leaf spot of muscadines may be effectively controlled. In this work a 4–5–50 Bordeaux spray was applied at intervals of about 2 weeks, and so timed that the first application preceded the first heavy ascospore discharge in the spring. The exact date for this application was obtained by a close check on the state of development of the perithecial material in the field, and when possible to do so, each subsequent spray was applied just preceding a rain.

Spraying may be discontinued after cessation of the 4–6 weeks' ascospore discharge.

The writer has no information on the degree of control possible through strict adherence to sanitary vineyard practices, but, knowing the nature of the pathogen's life cycle, one would be justified in recommending the destruction, either by fire or deep plowing, every fall, all leaves throughout the vineyard. If the leaves are turned under, one should be careful to avoid uncovering them while planting a cover crop. In the experience noted above, only 4 sprays were used, but the schedule was designed for muscadine black rot, the pathogen of which apparently has a less extended period of ascospore discharge in the spring than does *Mycosphaerella angulata.*

DICUSSION

The fact that muscadines as a commercial crop have not heretofore been of much economic importance is probably the principal reason they have received so little attention from plant pathologists. From a cursory examination of numerous plantings in Georgia, it is evident that vineyard losses are considerable in certain seasons as regards both foliage diseases and berry rots. However, since the crop has not been properly evaluated in past years, no monetary comparison can be made between the disease-induced losses of bunch grapes and muscadines.

Although the results of the present investigation do not justify a comprehensive discussion of muscadine diseases, it is not premature to add that muscadines are subject to attack by several diseases other than angular leaf spot. The fact that bunch grape and muscadine culture are coextensive in the Southeast probably in a great measure accounts for the assumption that both are subject to the same diseases. Results of investigations, not yet complete, on other diseases of muscadines indicate an interesting parallel between those of muscadines and bunch grapes. For the most part, however, the pathogens appear to be specifically distinct.

Muscadines are strictly dioecious, and this fact, combined with the observation that individual male vines show variable evidence of infection by the several prevalent diseases, is suggestive that care in the selection of breeding stock may yield resistant varieties of superior quality. In this connection the writer has had occasion to check back on the original male parent used in the production of several of the better quality varieties introduced by this Station and found this parent extremely susceptible to both angular leaf spot and muscadine black rot. Other male vines, apparently more resistant might have yielded more resistant progenies had disease resistance been considered during the breeding program.

SUMMARY

The symptomatology and etiology of a foliage disease, herein designated angular leaf spot of muscadines, has been studied over a period of two seasons. The lesions first appear as small chlorotic areas on the upper leaf

surface. Rapid development results in the production of angular lesions, visible on both leaf surfaces, surrounded by halos on the upper surface. The halos tend to disappear with age. The disease is probably coextensive with muscadine culture in the Southeast.

The pathogen, heretofore known as *Cercospora brachypus* Ell. and Ev., was observed producing spermogonia and perithecia in addition to conidia. Apparently, the perfect stage has not been previously described and is herein designated **Mycosphaerella angulata** n. sp.

Results from a single season's work indicate that angular leaf spot may be controlled by properly timed applications of a 4–5–50 Bordeaux spray. It also is suggested that strict vineyard sanitation may prove an important supplement to spraying.

On the basis of observation alone, it is suggested that a judicious selection of resistant breeding stock may be expected to produce more resistant progenies of superior-quality muscadines.

GEORGIA EXPERIMENT STATION
EXPERIMENT, GEORGIA.

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WIND DISSEMINATION OF ANGULAR LEAF SPOT OF COTTON

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Wind-blown dust as a disseminating agent of the angular-leaf-spot bacterium (*Phytomonas malvacera* (E. F. Sm.) Com. S.A.B.) probably has received less attention than wind-blown rain[1] because dust seldom plays an important role in the dispersal of the pathogen. Dust dispersal of the organism, however, has previously been reported from Oklahoma.[2] In Arizona, where these studies were made, the situation was perhaps more definite, since attendant circumstances made the prevailing conditions fairly comparable to a planned scientific experiment.

An experiment planned to demonstrate the natural spread of the angular-leaf-spot germ by wind-blown dust would necessitate, besides the moving dust, (a) a large plot or field of infected cotton as a source of inoculum, and (b) a noninfested field to which the bacterium could be carried. Such an experiment might involve also the wounding of the healthy plants at the proper time to make sure of infection, in case the bacterium reached the host. Since flowing irrigation water, in contact with infected plants, carries the germ of angular leaf spot and black arm, and may subsequently spread the disease to healthy plants that it laves, fields of infected and healthy plants should be individually irrigated with water from separate clean sources. In location, fields of healthy plants on the one hand and diseased ones on the other should bear the proper relation with respect to the prevailing wind, so that the dust will be carried from the former to the latter and not contrariwise. In order properly to evaluate the results, an isolated control field planted with seed of the same variety of cotton would undoubtedly be prepared. The specified conditions are not usually encountered in commercial cotton growing; indeed, if the conditions were planned by a careful investigator, the experiment would probably fail because nature cannot be depended upon to provide the dust storm, even in Arizona. Yet, exactly the described conditions occurred in southern Arizona in 1940, including a local hailstorm that conveniently wounded the healthy cotton plants that were to receive the inoculum. The area subjected to dust and hail is here designated as District 1; that containing the control plantings, as District 2 (Fig. 1).

GENERAL CONDITIONS

Climatic conditions in both districts were essentially similar, since the two districts are less than 15 miles apart in a direct line. The prevailing winds of summer are from the east and southeast. Precipitation is low and irrigation is largely depended upon for water supply. Showers are some-

[1] Faulwetter, R. C. Dissemination of the angular leaf spot of cotton. Jour. Agr. Res. [U. S.] 8: 457–475. 1917.

[2] Rolfs, F. M. Dissemination of the bacterial leaf spot organism. Phytopath. 25: 971. 1935.

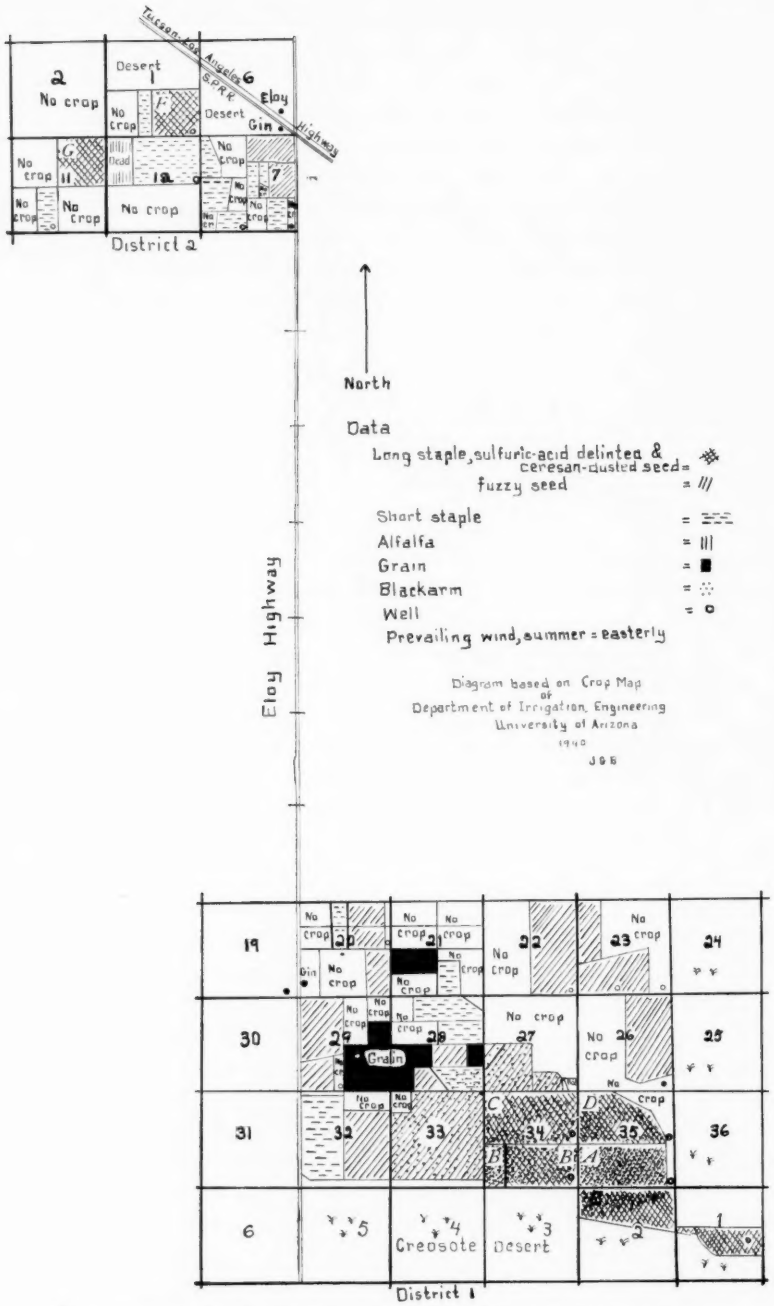

FIG. 1. Map showing location of cotton fields studies in Arizona.

times local, reaching only a few hundred acres; this is particularly true of hail storms. The soil has a fine texture and, when thoroughly dry, forms a powdery dust.

The districts were without serious pathological complications, although alternariosis had caused approximately as much damage as angular leaf spot and black arm, in District 1, and considerable damage in District 2. Other diseases of cotton, including verticillium wilt, root knot, and Texas root rot were absent, excepting a very small area of root rot in one field (Field D).

FIELDS STUDIED

The fields that supplied the inoculum consisted of approximately 320 acres and 80 acres, respectively. They were planted with nontreated seed of the SXP variety and irrigated only with water from wells in the same field. The larger field (Fig. 1, A, S½ of Section 35) was separated from adjacent cotton fields on 3 sides by dry, usually dusty, roadways, 60 to 100 feet wide, and on the fourth side it was bordered by desert. The smaller field (Fig. 1, B′, W½ of the SW¼ of Section 34) likewise had broad, dry roadways on 3 sides, but was not separated from cotton on the east. Of the fields of cotton that received the inoculum (Fig. 1, D, approximately the N½ of Section 35; Fig. 1, C, the N½ of Section 34; Fig. 1, B, the eastern part of the S½ of Section 34; and Fig. 1, E, part of the N½ of Section 2), all were isolated one from another by broad, dry roadways, excepting Field B adjoining on the west, without barrier, Field B′. For convenience the fields referred to in this paragraph are collectively designated as District 1 (Fig. 1).

Cotton fields regarded as controls for the purpose of comparison were located in District 2 (Fig. 1), approximately 11 miles north and 4 west of the district mentioned in the preceding paragraph. They were the SE¼ of Section 1 (Fig. 1, F) and the NE¼ of Section 11 (Fig. 1, G) west of Eloy. Field F was separated by a roadway of standard width from desert on the east and from fields of short-staple (Acala) cotton on the southeast and south; on the west a field of Acala cotton adjoined the field of SXP variety without a barrier. To the north lay the desert. Field G adjoined a roadway on the east and was completely isolated from other cotton fields, excepting the southwest corner, which lay opposite a small field of Acala cotton. These fields were planted with sulphuric acid-delinted, Ceresan-dusted cotton seed from the same lot used in the south district.

All fields directly concerned in these studies were planted with the SXP variety of long-staple cotton. Delinted seed was treated by machine with concentrated sulphuric acid, washed, dried, and dusted with 2 per cent Ceresan. In the process, all light-weight seeds were separated from the heavy seeds. "Fuzzy seed" (a term here applied to the seed as it comes from the gin) was not treated. The planting rate for the delinted seed was 9 lb. per acre and for the fuzzy seed 25 lb. per acre. All fields were watered from individual wells. The fields in the southern district (Fig. 1, Sections 34 and 35) had never before borne a crop, having been broken from the desert last year. The larger, A, of the two fields from untreated seed was the sole source of inoculum east of the fields that became infested later.

Only two species of plants other than cultivated cotton[3, 4] are known to be susceptible to the angular-leaf-spot bacterium, and neither species is found in this district.

Black-arm Infested Fields.—The study of the fields was occasioned by the appearance of the black-arm phase of angular leaf spot in cotton grown from acid-delinted and Ceresan-dusted seed, a condition so unusual as to excite comment. The angular-leaf-spot phase was practically absent. Cotton fields from delinted seed, in the northwestern part of the district (District 1, Fig. 1), were free from angular leaf spot when they were examined by the writer in the spring, but an occasional infected plant was found in fields planted with fuzzy seed. The first report of the presence of the disease in fields from treated seed came to the writer early in September, although farmers stated that black arm appeared in August. Examination of the fields verified the presence of the disease in destructive form. The following picture of the situation will be clearly understood by referring to the map (Fig. 1).

Plants in fields B and B′ were most affected by black arm. The stand was almost perfect, yet there were not enough bolls to justify harvesting. Individual plants in Field B showed black-arm lesions mostly extending from the middle, or a little below, to the top; nearly all the lesions were around wounds (Fig. 2, A, B).

The uniform distribution of black arm throughout Field B, as well as the location of the lesions on the infected stems, could not be reconciled with the suggestion of dissemination by irrigation water. In that case, infected plants are more numerous near the irrigation ditch and the stages of black arm are more advanced as compared with plants farther removed from the source of infection. Furthermore, resulting lesions are located at the base of the stem, since infection from irrigation water is limited to the seedling stage.

Field B′, planted with fuzzy seed and adjoining Field B without a barrier, had even fewer bolls than Field B. Individual plants mostly showed lesions from the middle part to the top, but an occasional plant (Fig. 2, C, D) had black-arm lesions at the base.

Field A, planted with fuzzy seed, had a stand like that in Fields B′ and B, but matured very little cotton. Black-arm scars were found on the bases of some plants as well as around wounds on the stems. The wounded plants were more numerous in the western part of the field.

Field D, planted with acid-delinted and Ceresan-dusted seed, yielded more cotton than the fields previously described. Black-arm lesions were located mainly from the middle, or somewhat below, to the top of the plants. Like the other affected fields, Field D showed loss of bolls caused by the angular-leaf-spot bacterium (Fig. 3, D) and by Alternaria. Wounded plants were most numerous in the southern and western parts of the field.

[3] Brown, J. G., and Frederick Gibson. A new host for *Bacterium malvacearum*. Phytopath. 13: 455–457. 1923.

[4] Palm, B. T. Eriodendron as host of *Bacterium malvacearum*. Phytopath. 22: 867–868. 1932.

FIG. 2. A. Field (Fig. 1, District 2, F) of S×P cotton taken after first picking. The field was planted with sulphuric acid-delinted and Ceresan-dusted seed, from the same lot used in Fields B, C, and D in District 2. There was no black arm in this field. B. Field of S×P cotton (Fig. 1, District 1, D) grown from sulphuric acid-delinted and Ceresan-dusted seed. Black arm appeared late in this field and was worse on the south side adjacent to a field (Fig. 1, A) grown from untreated seed. C. Lower parts of stems of cotton plants, left, from clean Field F; right, from black-arm-infested Field B′. The dark spot near the base of the plant on left is a small leaf and its shadow. a. Inactive black-arm lesions from seedling stage. b. Black-arm lesions higher on stem; arrow points to former attachment of black-arm-affected branch. D. Upper parts of cotton stems illustrated in C. Stem on left, grown from treated seed, is clean. l. Extensive black-arm lesion on stem of plant from untreated seed; the dead bark is separated from the sound bark below and along the side by a deep crack. The top of the plant was killed by black arm. E. Cotton plant grown from untreated seed (Field B′). The plant was stunted and apparently had been affected with black arm since the seedling stage. a. Inactive basal stem lesion from which the angular leaf spot bacterium was isolated. b, c. Infection spots linking basal lesion with black-arm-blighted top, d.

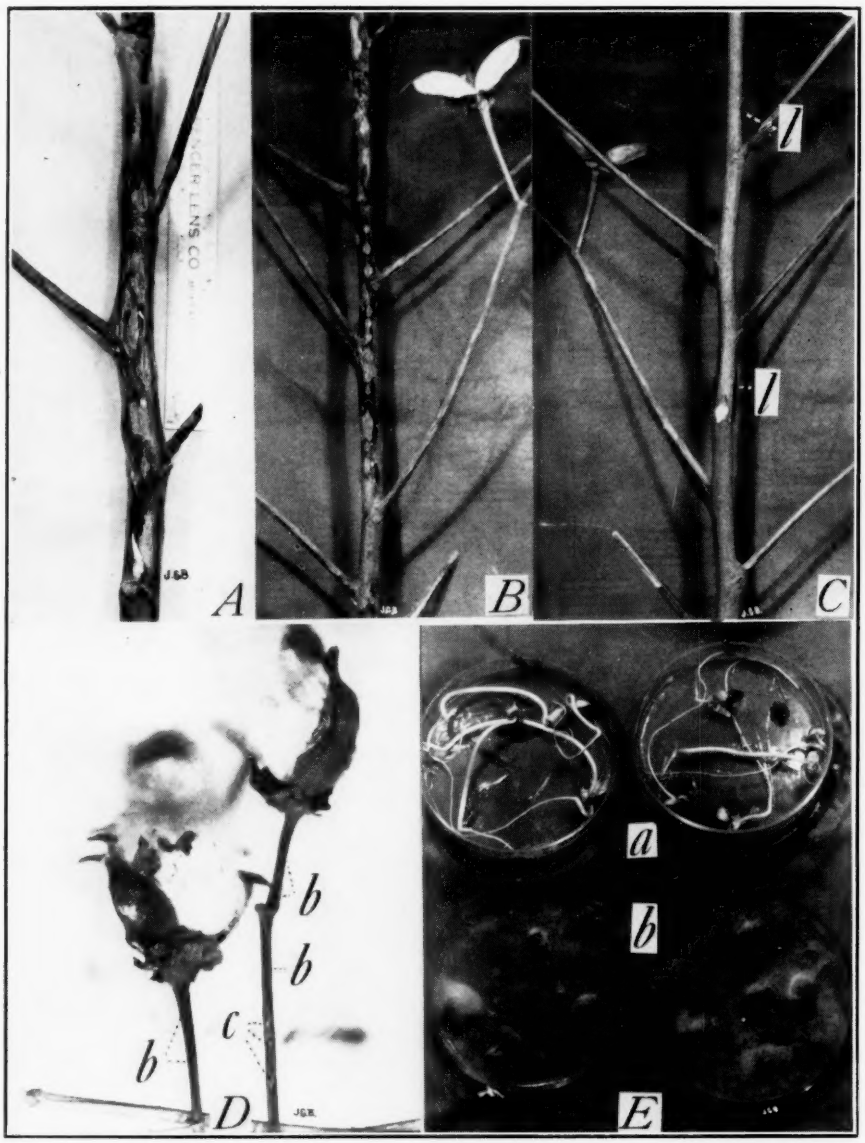

FIG. 3. Piece of cotton stem from Field B (Fig. 1) showing hail wounds, each wound more or less surrounded by a black-arm lesion. B. East side; C, west side of part of the main stem of a cotton plant from Field B (Fig. 1); l, margin of lesion mainly on opposite side of stem. This plant was typical for the hail-injured, black-arm-affected plants in the fields from treated seed. D. Cotton bolls and pedicels with glistening black-arm lesions, b, and thin, pale, dried flecks of bacterial ooze, c. E. Cultures of cottonseeds from the lot left after planting the fields studied: a. Sulphuric acid-delinted and Ceresan-dusted seeds. b. Untreated, fuzzy seeds. Cultures of the treated seed usually were clean; cultures of the untreated seeds gave mold and other fungi, also colonies of bacteria, including *Phytomonas malvacera*.

Field C, planted with sulphuric-acid-delinted, Ceresan-dusted seed, showed plants with less black arm than those in Fields B and A and also produced more cotton. Black-arm lesions were mainly from the middle region of the plants to the top; they were associated with wounds.

In Field E, planted with acid-delinted, Ceresan-dusted seed, the plants were affected by black arm only on the north and west sides of the field, in a triangular area, with the apex in the northeast corner and the base along the west side. In this area plants had black-arm lesions that extended from the middle region of the stem toward the top and were associated with wounds.

The presence of wounds on the cotton plants is explained by the occurrence of a local hailstorm on August 13. Serious wounding of cotton plants resulted in Fields A and E and westward at least to Section 32, and northward over District 1. On August 20, a windstorm, violent enough to tear leaves from plants and hurl them over a 100-foot wide roadway, swept the same area. Both storms very evidently came from the east, the generally prevalent direction of summer storms in southern Arizona. Local residents state that unlatched east doors of their homes were blown inward; hail wounds resulting from the first storm were mostly on the east sides of the main cotton stems. (Fig. 3, B, C).

The "Control" Fields.—For comparison with the cotton crop in District 1, two fields may be used that were planted with sulphuric acid-delinted, Ceresan-dusted seed from the same lot used in Fields B, C, and D. That these fields (Fig. 1, F, G) were partly isolated from others of long-staple cotton, may be seen at a glance, although both were adjacent to plantings of the Acala variety which is less susceptible to black arm. No black arm was found in Fields F and G. Four bolls affected with rot induced by the bacterium of angular leaf spot were collected in Field F, of which 3 showed insect punctures in the decayed spots; 5 similarly infected bolls were found in Field G. The bolls were mostly "top crop" and the infection therefore late. Many bolls had been killed by an Alternaria.

THE SEED

The culturing of cottonseed from the lots used in planting the fields was carried out. Plenty of the acid-delinted seed was available, but the only fuzzy seed left after planting was a small quantity removed from the planter and dumped on the ground. Representative samples of the cultures of the delinted seeds (Fig. 3, E, a) and of the fuzzy seeds (Fig. 3, E, b) are illustrated. The acid-delinted and Ceresan-dusted seeds usually gave no organisms whatever, although some seeds that were cultured were obtained from the dust on the ground where they had spilled from torn sacks; in no case did cultures of the delinted, dusted seeds give a yellow bacterium. The fuzzy seeds, as usual, gave a number of fungi and a pale yellow bacterium that became a deeper yellow as it grew older. This was the bacterium of angular leaf spot and black arm, *Phytomonas malvacera,* as subsequently proved by further planting, inoculations, and reisolation.

DISCUSSION

From the evidence presented by plants in the fields described above, it is clear that primary (seed-derived) infection with the angular-leaf-spot and black-arm germ existed in Fields A and B′; likewise clear that such infection did not occur in Fields B, C, D, and E. Supporting evidence are the healed black-arm lesions present on the bases of plants in Fields A and B′; also, lesions in the fields planted with acid-delinted seed and located in and above the middle region of the plants, a condition possible only in late infection.

It is quite probable that infected leaves fell to the ground and became broken and mixed with the dust in the fields first infested. The hailstorm of August 13, which severely wounded the growing plants in all fields mentioned, was followed by the inoculum-laden dust storm of August 20. The most abundant inoculum thus transported reached Field B, directly west of Field A (fuzzy, untreated seed); also, some inoculum was carried into the north side of Field E in an area widening westward; some into Field D, the heavier dose reaching the south side, and some into Field C, likewise most severely infested on the south side. Field B′ probably contributed little if any inoculum to Field B, but cotton growing farther west, in the direction of the moving dust, may have received inoculum from Field B′, since black arm was present in Section 33 (Fig. 1).

The effectiveness of hail in increasing infection with the angular-leaf-spot bacterium is very well illustrated in these studies (Fig. 2, A, B). Properly timed with moving, inoculum-charged dust, a hailstorm apparently is important in the spread of angular leaf spot and black arm. In the fields here discussed, the infection with black arm was very uniform in development over an area 2 miles long, east to west (Fig. 1, south parts of Sections 35 and 34), whereas Faulwetter[5] observed the spread of inoculum by wind-blown rain over a much smaller area. Even allowing for the comparatively limited source of inoculum in Faulwetter's studies, wind-blown dust must be regarded as a more effective carrier. Comparison of the studies in Arizona with Rolf's[6] observations serves to emphasize the destructiveness of hail in combination with moving dust. Rolf observed that "a single whirlwind scattered the infected dry leaf material over 100 acres in 20 minutes." In the Arizona storm preceded by hail, the infected material started disease over more than 1,000 acres.

The almost total absence of the angular-leaf-spot phase of disease following the hail and dust storms is significant. Although the leaves of cotton plants in District 1 must have been wounded by hail and contacted by the inoculum-laden dust, almost no infection resulted. Presumably, the surface of the leaves exposed to the air dried before the bacterium of angular leaf spot could start disease, whereas, the juicier wounded stems afforded sufficient moisture long enough to permit the growth and multiplication of the organism. Rapid drying of the surface of susceptible plant parts may

[5] See footnote 1.
[6] See footnote 2.

explain the comparatively rare dissemination of angular leaf spot by wind-blown dust.

SUMMARY

A field of 240 acres of the black-arm-susceptible SXP cotton plants grown from sulphuric-acid-delinted and Ceresan-dusted seed was extensively and uniformly infested and adjacent fields less affected, in September, with the black arm phase of angular leaf spot. The field with most severely infected plants lay directly west of a half-section of land planted with nontreated or fuzzy seed, and it adjoined on the west, without barrier, an 80-acre field also planted with untreated seed.

A field of cotton from delinted and dusted seed that lay north of the half-section from nontreated seed contained more diseased plants on the side adjacent to the latter field. The ranch south of the half-section from fuzzy seed had black arm only in plants on the side of the ranch next to the half section.

Although the stand of cotton in all the fields was good, evidence existed that black arm was present in the fields from fuzzy seed and not present in the fields from delinted and dusted seed in the seedling stage of the crop. This evidence agrees with the observations made in the spring a few miles farther north and in the same district.

No source of black-arm inoculum existed other than the cultivated cotton. The inoculum could not have been carried into the fields from treated seed by irrigation water, for the fields were individually irrigated from deep wells and separated one from another by dry roadways; furthermore, black arm was quite uniformly distributed over the fields from treated seed and fairly uniformly developed in the plants from treated seed, rather than more extensive and in later stages in plants near irrigation ditches and in earlier stages in plants farther removed from the ditches.

Cultures of acid-delinted and Ceresan-dusted seed from the same lot planted in the fields from treated seed usually gave neither bacteria nor fungi, and in no case did they give a yellow bacterium. Cultures of the fuzzy seed from the same lot planted in the fields from untreated seed gave a luxuriant growth of fungi and also yellow colonies of bacteria that were proved to be the angular-leaf-spot (black-arm) germ.

The cotton fields were swept by a hailstorm on August 13 and by a dust storm one week later. The general direction of the storms was east to west. Prior to the storms no destructive action of black arm had been noticed. The hailstorm wounded the cotton plants and the dust storm disseminated the inoculum of black arm. By September, infection with the bacterium of black arm was uniform throughout the fields from treated and untreated seed.

Cotton fields here used as checks or controls against the black-arm infested fields were planted approximately 12 miles distant from the latter, by the same farmers, with sulphuric acid-delinted and Ceresan-dusted seed from the same lot used in the infested fields, in like soil, under similar con-

ditions excepting hail injury and subjection to violent dust storm. The control fields were isolated from other fields of the black-arm-susceptible, long staple (SXP) cotton. No black arm was found in the control fields.

Conclusions were: (a) that the black-arm inoculum was carried in dust from the half section of land bearing infected cotton plants reared from fuzzy seed, that the heaviest dose of inoculum reached the cotton field on the west, but that the inoculum-bearing dust spread laterally westward in a somewhat fan-shaped belt to reach bordering fields on the north and on the south while inoculum from the smaller field reared from fuzzy seed was also carried westward at the same time; and (b) that since the area of cotton directly affected included more than two sections of land, wind-blown dust following a hailstorm was most efficient in disseminating the bacterium of angular leaf spot.

Pursuance of these studies was facilitated by the absence of all seed-borne diseases from the crop, except black arm, and the almost absolute freedom of the fields from diseases such as verticillium wilt, Texas root rot and root knot that are caused by soil-dwelling parasites. On the other hand, part of the damage attributed to black arm by farmers was caused by the air-distributed fungus, *Alternaria* sp.

As an addendum for any cotton grower into whose hands, perchance, this paper may fall, it is pointed out that, although the combination of meteorological events that contributed to the heavy loss (estimated to total $60,000 for the two farmers concerned) is not a common occurrence, mixed planting of treated and untreated cotton seed is unwise.

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PHYTOPATHOLOGICAL NOTES

A Xylaria Pathogenic to Ginkgo biloba L. Seeds.[1]—In the course of a pathological survey of the Morris Arboretum, Philadelphia, Pennsylvania, in the summer of 1938, a species of *Xylaria*, tentatively identified as *X. longeana* Rhem, was isolated from dying and dead branches of a staminate specimen of *Ginkgo biloba* L. Experimental inoculations of seeds, seedlings, and branches of mature trees were studied to determine the pathogenicity of the fungus. The results with seedlings and mature trees are as yet incomplete.

Seed was collected in the fall of 1938 and of 1939. The outer fleshy layer was removed by washing and the seed then stored in a cardboard carton in a refrigerator at 40° F. The seeds were planted the April following collection, one-half inch deep in native clay loam in twelve-inch pots, and kept under ordinary greenhouse conditions. The inoculation material consisted of the fungus, which was growing in steam-sterilized wheat. This was incorporated into the soil at the time the seeds were planted. Since no significant difference was noted in the results observed with sterilized and unsterilized soils in the first series, only the latter were used in subsequent experiments.

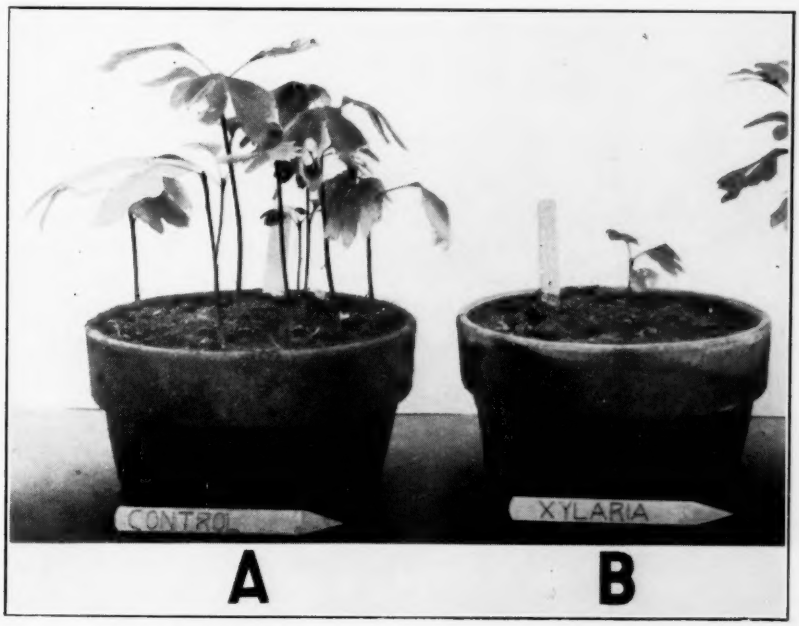

FIG. 1. Seedlings 5 months after planting. A. Control. B. Inoculated. $\times \frac{1}{7}$.

Good emergence occurred in the control pots over a period of ten weeks, while the seedlings in the inoculated pots were badly stunted (Fig. 1). In the inoculated pots the seeds that did not germinate and the seedlings that

[1] Acknowledgement is made to Dr. Harlan H. York for helpful suggestions in the course of this work.

TABLE 1. *Percentage emergence of seedlings of Gingko biloba in sterilized and nonsterilized soil certain pots of which were inoculated and others left as controls*

Year	Soil	Culture	Emerged
			Per cent
1939	Nonsterile	Controls	32.0
	Sterile	Controls	36.0
	Nonsterile	Inoculated	2.0
	Sterile	Inoculated	2.0
1940	Nonsterile	Controls	55.4
	Nonsterile	Inoculated	2.5

failed to emerge were covered with thick, black mycelial masses resembling stromata (Fig. 2, D). From such seeds fruiting bodies of the *Xylaria* had pushed up above the surface of the soil. Removal of these masses of mycelium from the stony layer of the seed revealed black zone lines[2] and spots (Fig. 2, E), which were extended through the stony layer into the papery layer of the testa (Fig. 2, F and G). Seeds that failed to germinate or were

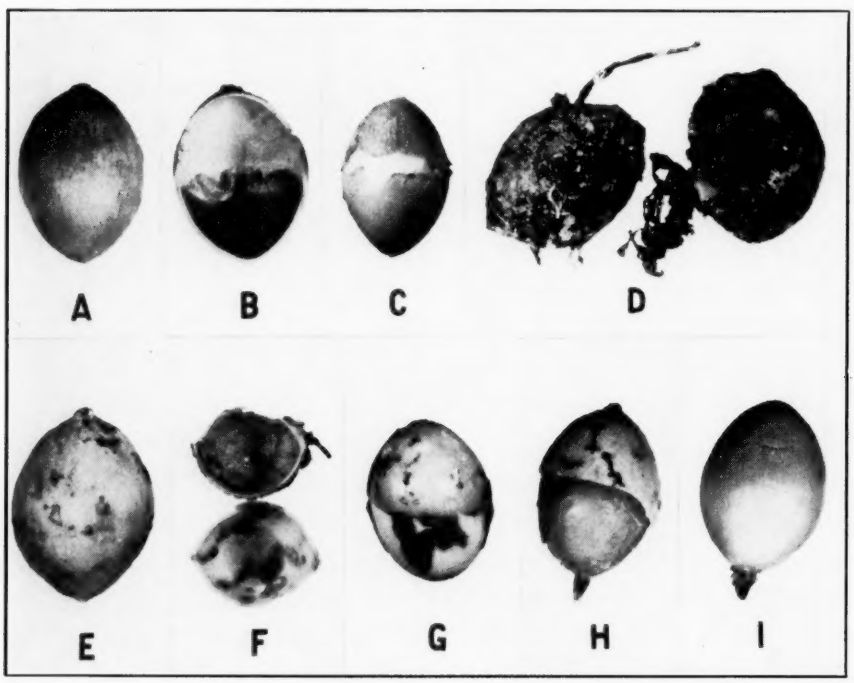

FIG. 2. A. Normal seed with stony layer unbroken. B. Normal seed showing inside appearance of stony layer. C. Normal seed with stony layer removed. D. Seeds from inoculated soil; black mycelium and fruiting body of *Xylaria* on seed. E. Mycelium scraped from seeds in D, showing black zone lines on outer surface of stony layer. F. Seed from D, showing inside appearance of stony layer. G. Seed from D with stony layer removed. H and I. Seeds from inoculated pots, killed soon after germination. All ×1.

[2] Hilborn, M. T. The Anatomy of a black zone caused by *Xylaria polymorpha*. Phytopath. **27**: 1177. 1937.

killed in the preemergence stage (Fig. 2, H and I) were cultured and the *Xylaria* reisolated.—SPENCER H. DAVIS, JR., and JOHN B. HARRY, The Morris Arboretum, Department of Botany, University of Pennsylvania, Philadelphia, Pa.

A Virosis-like Injury of Snapdragon Caused by Feeding of the Peach Aphid.—Snapdragon seedlings and cuttings grown for experimental purposes under glass at Los Angeles in the Spring of 1940 commonly showed an injury of the terminal growth, which resembled a virosis. Subsequently, the same injury was observed on *Antirrhinum* plants, growing both under glass and in a protected outdoor location in the San Francisco Bay area, and again in the Spring of 1941 under glass in Los Angeles. Careful examination of the plants showed the presence of no pathogen and only occasional aphids or their cast skins. It appeared that the disease was caused by a virus or resulted from the feeding of the aphids.

The genetical value of some of the plants made desirable their vegetative propagation, but the cessation of growth of diseased shoots would have made this unsuccessful if a virus had been involved. Tests were, therefore, undertaken to determine the cause of the disease.

The injury has been observed occurring naturally on *Antirrhinum majus* L., *A. speciosum* Gray, *A. nuttallianum* Benth., *A. glandulosum* Lindl., *A. virga* Gray, *A. molle* L., and *Linaria dalmatica* Mill. The symptoms were essentially alike on all these species.

The most characteristic feature of the injury was that apical growth was checked and each rosette of leaves, when viewed from above, had a pale yellow to white center with a periphery of green, or of green mottled or spotted with yellow. Stem tips thus injured often became brown and died, but sometimes resumed growth after a time (Fig. 1, C) or gave rise to new lateral branches (Fig. 1, D).

The leaves were greatly reduced in size and frequently were rolled, dorsally curled, and laterally distorted (Fig. 1, A). Sometimes there were yellow or white spots 1 mm. in diameter in the laminae of apical leaves, particularly near the veins (Fig. 1, B). The midrib and principal veins were yellow to white and the pallor extended somewhat to the surrounding tissues, particularly at the leaf bases. Rugosity occurred only in cases in which yellow areas partially or wholly surrounded a green island. Injured leaves have never been observed to recover. Sometimes, particularly in a tetraploid and in a double-flowered diploid variety of *A. majus*, persistent, bright yellow, circular spots up to 5 mm. in diameter developed in the basal leaves.

The injury was found to be due to feeding of the peach aphid (*Myzus persicae* Sulz.[1]) Previously healthy snapdragon seedlings showed typical injury of the leaves on which small numbers of these aphids had fed. New foliage formed on either a continuation of the same axis or its laterals,

[1] Determination by E. O. Essig.

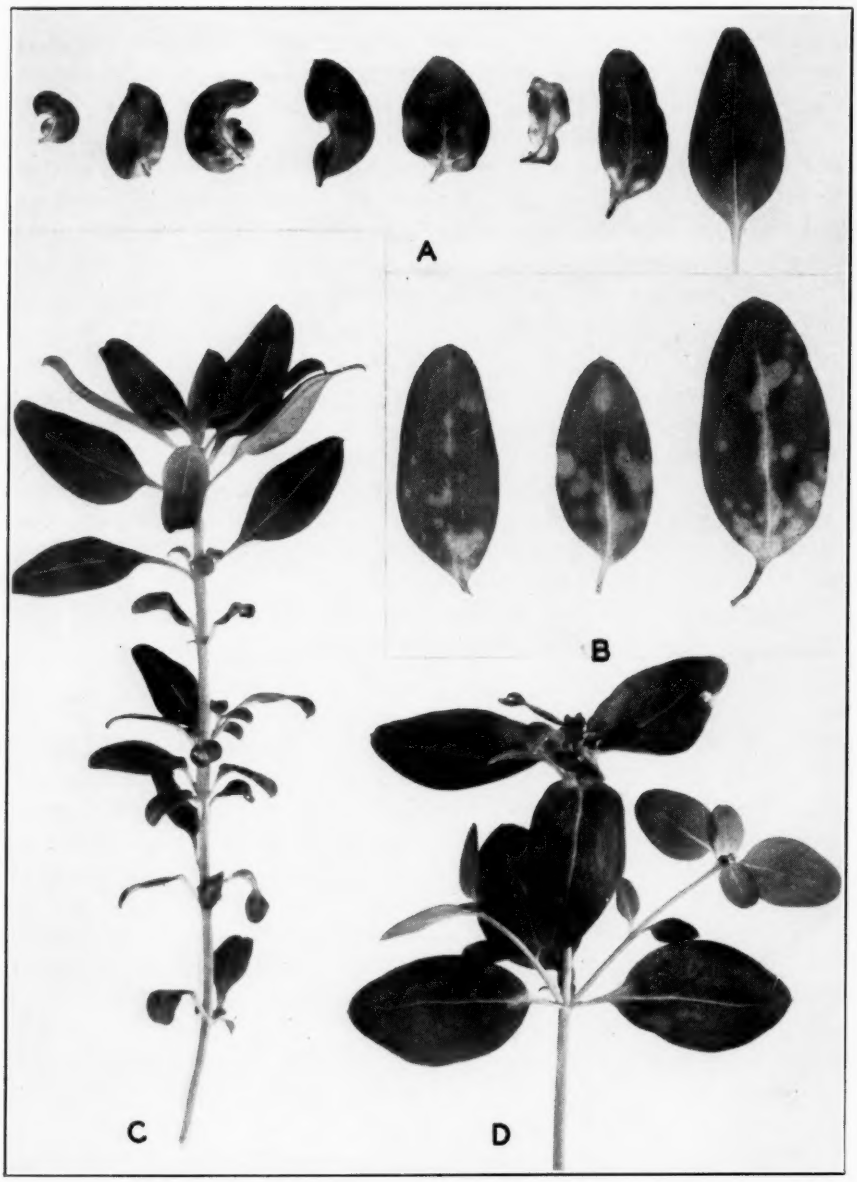

FIG. 1. Virosis-like injury to *Antirrhinum* spp. caused by feeding of *Myzus persicae*. A. Apical leaves of *A. majus*, showing distortion and discoloration; check at right. B. *A. speciosum* leaves with characteristic pallid spots. C. *A. majus* with stunted and distorted lower leaves and normal terminal growth produced after removal of aphids. D. *A. majus* with development of growing point completely checked by aphids and normal new growth arising laterally following removal of aphids.

following the removal of the aphids, was entirely normal under environmental conditions in which infested plants contined to show symptoms (Fig. 1, C). Peach aphids from a non-viruliferous line that had been main-

tained for many generations on turnip gave symptoms on *Antirrhinum* identical to those caused by insects taken directly from diseased snapdragons. Plants showing the injury outdoors developed healthy new growth when freed of aphids and either taken into a glasshouse or kept outdoors; plants that had not been freed of aphids contined to show symptoms. Plants that had recovered did not show symptoms again in 4 months time. Only leaves on which aphids had fed have showed these symptoms. Plants with several shoots have shown symptoms only on the specific branch on which the insects have fed.

Attempts to transmit a virus, by the carborundum method, from diseased snapdragons to Turkish tobacco, *Nicotiana glutinosa,* spinach, tomato, cucumber, and snapdragon were unsuccessful.

It is concluded that the injury was caused by feeding of the peach aphid, probably resulting from some toxic secretion of the insect. The aphid is so toxic that a few insects can cause a surprising amount of injury, particularly because they collect at the tender growing points. Although the aphid can build up high populations when caged on snapdragon, it has not been seen to do so on unprotected plants. Plants with both aphids and the injury they cause have consistently recovered when planted in the field under southern California conditions.—KENNETH F. BAKER and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

Technique for Artificially Feeding Scolytus multistriatus and Saperda tridentata Spores of Ceratostomella ulmi and Other Substances.—It is now established that *Ceratostomella ulmi* (Schwarz) Buisman is disseminated chiefly by *Scolytus scolytus* Fab. and *S. multistriatus* Marsh. in Europe and by the latter species in the United States. Grossmann[1] and Betrem[2] determined that spores of *C. ulmi* were carried internally and externally by *S. scolytus.*

Collins *et al*[3] reported the isolation of *Ceratostomella ulmi* from adult *Scolytus multistriatus,* captured while crawling on elm logs in the field. But no record has been found of work to determine whether the fungus is carried internally or eliminated in the feces by *S. multistriatus.*[4] To study this and other relationships between the beetle and fungus, a technique was devised for artificially feeding the beetles under conditions precluding external transfer of the fungus or other substances from the mouthparts to the posterior portion of the body and fecal pellets.

[1] Grosmann, Helene. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Zeitschr. f. Parasitenk. **3**: 57–102. 1931.

[2] Betrem, J. G. De Iepenziekte en de Iepenspintkevers. Tijdschr. over Plantenziekten **35**: 273–288. 1929.

[3] Collins, C. W., W. D. Buchanan, R. R. Whitten, and C. H. Hoffmann. Bark beetles and other possible insect vectors of the Dutch elm disease, *Ceratostomella ulmi* (Schwarz) Buisman. Jour. Econ. Ent. **29**: 169–176. 1936.

[4] Since the preparation of this manuscript, Parker *et al.* have reported the isolation of *C. ulmi* from the gut of *Scolytus multistriatus.* Parker, K. G., Philip A. Readio, Leon J. Tyler, and Donald L. Collins. Transmission of the Dutch elm disease pathogen by *Scolytus multistriatus* and the development of infection. Phytopath. **31**: 657–663. 1941.

Active, recently emerged adults, free of *Ceratostomella ulmi*, were stuck to the ends of tapered matchsticks with cellulose glue. The legs were fastened to the end of the stick, leaving the head and the posterior part of the abdomen free.

A small stand (Fig. 1, A) was constructed from 2 pieces of wood. The matchstick with the attached beetle was stuck in a hole near one end of the base. A glass tube was mounted on the end of another matchstick, inserted in a radio binding post. The stick was adjusted so that the head of the beetle was just inside the end of the tube (Fig 1, B). The tubes were loaded with the various substances to be ingested. Air currents and dust were kept from the apparatus by a celluloid cover. Beetles freely ingested food for as much as 15 days and water and other substances for several days while mounted on the matchsticks. In other experiments predetermined small quantities of spore suspensions were injected directly into the mouths of the beetles with the aid of a micromanipulator and a micro-pipette.

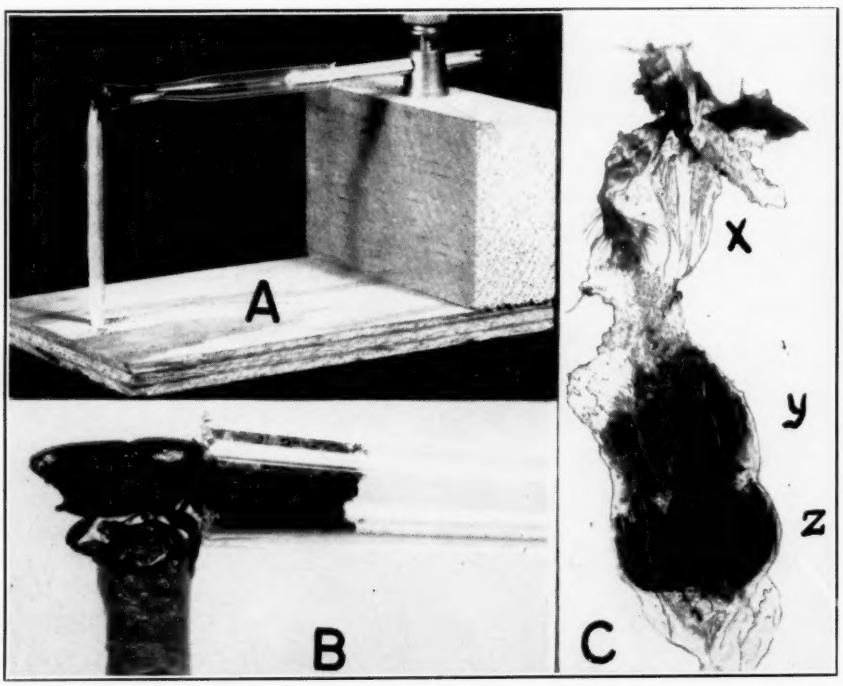

FIG. 1. A. Apparatus for artificial feeding of *Scolytus multistriatus*. ×¾. B. A beetle with legs glued to the end of a stick and head inserted in feeding tube. ×4. C. Foregut of *Scolytus multistriatus* that had fed on spore suspension of *Ceratostomella ulmi* mixed with elm bark: x, oesophagus; y, ingested food in crop; z, proventriculus. ×60.

Coremia of *Ceratostomella ulmi*, spore suspension of that fungus, spore suspension of *C. ulmi* mixed with finely ground elm bark, water, arsenic, and a solution of Neoprontosil were fed to the beetles. Feeding was readily observed under a binocular microscope.

The entire digestive tract was dissected out of several beetles and observed under the microscope.[5] Ground bark, similar to that fed the beetles, was found in all parts of the gut and in fecal pellets voided by the beetles. Beetles usually commenced to discharge such pellets about 1½ hours after they began to feed. Ingested material was observed in the crop (Fig. 1, C, y) of beetles that had fed for short periods. In beetles that had not fed, the entire gut was empty except for the rectal area, which always contained small white pellets of material that was not identified.

Pellets of excrement were obtained from the feeding beetles under conditions precluding their accidental contamination by *Ceratostomella ulmi*. The feet were fastened and could not accidentally transfer contaminated particles from the heads to the posterior parts of the abdomens. The entire posteriors of the beetles were frequently surface-sterilized by brushing with 95 per cent alcohol. The alcohol treatment did not interfere with feeding or prevent elimination of feces.

Adult *Scolytus sulcatus* Le C. and *Saperda tridentata* Oliv. have been fed successfully by the same method.—W. D. BUCHANAN, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, and CURTIS MAY, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

Aecidium gossypii, the Aecial Stage of Puccinia bouteloуae.[1]—Connection has been established between *Aecidium gossypii* and a *Puccinia* on *Bouteloua* spp. Observations had indicated that there might be a connection between the uredial and telial stages of the rust on the grasses and the aecia on cotton. The connection was finally established by inoculating cotton with telial material from *Bouteloua aristidoides* and *B. barbata* and then reinoculating these 2 grasses with the resulting aecia on cotton. That cotton is the aecial host for the rust on the *Bouteloua* in nature is indicated by field observations.

In the late summer and fall of 1940 there were heavy epidemics of cotton rust in several widely separated localities of Arizona. There was particularly good opportunity to study the rust at Hidden Valley (Pinal County) and to observe the native vegetation growing on the desert immediately adjacent to cotton fields. In one field especially there was no possibility that the rust had been carried over on some other crop plant, because the field was

[5] The writers are indebted to R. T. Webber of the Bureau of Entomology and Plant Quarantine for his assistance in dissecting the gut from a number of beetles.

[1] Cooperative investigations between the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station. Paper No. 1910 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. The field work was done in the course of the writer's regular duties at the U. S. Dept. of Agriculture field station at Sacaton, Arizona. The greenhouse and laboratory work was done at University Farm, St. Paul, Minn., while the writer was on leave from federal duties and held a research assistantship at the University of Minnesota.

The writer wishes to express his deep gratitude to William Q. Loegering, Agent in the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, stationed at University Farm, St. Paul, Minn., for cooperation in the laboratory and greenhouse work.

under cultivation for the first time, having been cleared of desert growth the previous year. The native plants were critically examined in the hope of finding the unknown telial host of the cotton rust fungus; two species of grass, *Bouteloua aristidoides* and *B. barbata*, were found heavily rusted. These observations were confirmed in the Continental area (Pima County) where the same two species of grass were found severely rusted adjacent to an infected field of cotton. The fact that the severity of rust on the grasses decreased with the distance from the cotton planting suggested a relation between the grass rust and that on cotton.

Fresh uredial and telial material was collected in September, 1940, and tentatively identified as *Puccinia boutelouae* (Jennings) Holw. This material was stored in a refrigerator; in March, 1941, telia that had overwintered on the desert were collected also. Both collections were used in inoculation studies at the University of Minnesota during April and May, 1941. Several species of *Bouteloua* (*B. aristidoides*, *B. barbata*, *B. curtipendula*, and *B. gracilis*) became infected when inoculated with the uredia collected in Arizona the previous fall. Cotton seedlings of the Acala variety, about 2 weeks old, were exposed separately to germinating telia from the fall and spring collections. The seedlings were placed in an incubator and telia that had been presoaked in water were spread on a wire screen above them. After 4 days the first series of seedlings was removed and another series placed under the telia for an additional 4 days. Relatively few pycnia appeared on the plants in the first series, but they were numerous in the second series, indicating that the telia had begun to germinate after approximately 4 days, and had thus caused a much heavier shower of sporidia to fall on the second series of plants.

The formation of aecial cups around the pycnial clusters was noticed about a week after the appearance of the pycnia. As soon as the first aecial cups had matured, plants of *Bouteloua* spp. were inoculated with the aeciospores; 8 days later uredia appeared. The characters of urediospores formed as a result of inoculating *Bouteloua* with aecial material were identical with those of urediospores on the grasses rusted in nature and with those of uredia formed on grasses inoculated with the fall collection of uredial material.

Aecidium gossypii, therefore, evidently is one stage of *Puccinia boutelouae*, as the telial material that caused the formation of aecia on cotton agreed in all respects with the description for this species. Furthermore, the uredial stage as found in nature and that resulting from inoculation of the grasses with the above-mentioned aecia on cotton also conformed in all respects to the description of the species. There seems no question, therefore, that the rust causing infection on cotton is *P. boutelouae*.

There is some question as to whether *Puccinia boutelouae* is a synonym of *P. vexans*, which also occurs on *Bouteloua*. The 2 species are quite similar morphologically, the most pronounced difference between them, according to

Arthur,[2] being the length of the teliospore pedicels. Nevertheless, the writer examined herbarium specimens labeled *P. vexans*, and they agreed with the description of *P. boutelouae*. It may be that there is only one rust or that *P. vexans* and *P. boutelouae* should be considered races of the same species. If it is concluded that there is a single species, the name *P. vexans* would have priority. This entire question, however, must await further observations, experiments, and examination of herbarium material.—JOHN T. PRESLEY, U. S. Field Station, Sacaton, Arizona.

Transmission of Chlorotic Streak of Sugar Cane by the Leaf Hopper Draeculacephala portola.[1]—The cause and the means of natural transmission of chlorotic streak of sugar cane have remained unknown since the disease was first differentiated by Wilbrink in Java in 1928. Most workers who have studied the disease have noted that the nature of spread in the field indicated possible transmission by an insect vector, although proof has been lacking.

Experiments on insect transmission of the disease were conducted in Louisiana by the writers during 1941 in an insect-proof greenhouse. Cages 3 ft. long, 3.5 ft. tall, and 2 ft. wide, with tight wooden bottoms, and covered with 40-mesh copper-wire cloth and very finely woven cotton cloth, were used on ant-proof benches. Into each cage were placed from 10 to 15 insect-free chlorotic-streak-diseased plants of the variety C.P. 29/320 growing in sterilized 4-in. clay pots in soil that had been subjected to flowing steam for 3 to 4 hours, and an equal number of insect-free healthy plants growing in steamed soil from cuttings treated with hot water at 52° C. for 20 minutes, which treatment eliminates the disease. In this note, the term "healthy" is used to refer to plants grown from cuttings so treated.

An average of 5 *Draeculacephala portola* per healthy plant was introduced into each cage and allowed to feed for from 7 to 14 days. The healthy plants were then removed from the cages in a closed room separated from the greenhouse and placed in other cages, where they were fumigated twice at intervals of 7 to 10 days before being returned to the greenhouse. In some instances the diseased plants were removed prior to introducing the healthy ones into cages with leaf hoppers that had been feeding for 2 to 4 weeks on diseased plants. The entire greenhouse was fumigated at intervals of 7 to 10 days.

A total of 490 healthy plants was exposed to the leaf hoppers in this manner, beginning in March, 1941, and continuing to late May. By September 1, 25 of these had developed typical leaf symptoms of chlorotic streak. Twenty of these were from cages containing both diseased and healthy plants, and 5

[2] Arthur, Joseph Charles. Manual of the rusts in United States and Canada. Purdue Research Foundation, Lafayette, Indiana, 1934. pp. 172–173.

[1] Although *Draeculacephala portola* Ball has previously been misidentified as *D. mollipes* (Say) feeding on sugar cane in the Gulf States, true *mollipes* appears to occur primarily in the northeastern United States and is not known as a pest of sugar cane in Florida or Louisiana.

were from those in which the healthy plants had been exposed to the leaf hoppers following removal of the diseased plants.

As controls, 60 healthy plants were caged with diseased plants, but without leaf hoppers, for the same period of time as those with the insects; between 300 and 400 healthy plants were grown in a bench adjacent to diseased ones in the greenhouse in which the transmission experiments were conducted; and 20 healthy plants were grown for 9 months in a bench of soil in the greenhouse, set alternately at 8-inch intervals with diseased ones, where their roots and leaves were in intimate contact. None of these has shown symptoms of chlorotic streak. In addition, 20 healthy plants have been grown in an insect-proof greenhouse for 2 years in contact with chlorotic-streak-diseased plants and have remained apparently healthy.

While these experiments are of a preliminary nature, they offer proof of transmission of this disease by *Draeculacephala portola*. This leaf hopper is usually more abundant in Louisiana than the total of all other sucking insects found on sugar cane, excluding mealybugs. The question of whether this leaf hopper or close relatives coexist with the disease is of interest. The genus *Draeculacephala* has been reported in Hawaii, Puerto Rico, Colombia, and Louisana, where chlorotic streak apparently spreads under natural conditions, but not in Java, where the disease was first described. Further experiments with this and other sugar-cane insects are in progress.—E. V. ABBOTT, Bureau of Plant Industry, and J. W. INGRAM, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Houma, Louisiana.

Susceptibility of Lee × Victoria Oat Selections to Loose Smut.—Since its introduction into the United States in 1927, the Victoria oat has been used extensively in crosses with other varieties for the development of disease-resistant strains. Several selections from these crosses have been named and distributed. Those from Victoria × Richland and Lee × Victoria have been especially promising for particular regions.

Victoria, with the exception noted below, has been highly resistant to smut. It was inoculated with many collections of loose (*Ustilago avenae* (Pers.) Jens.) and covered (*U. levis* (Kell. and Sw.) Magn.) smuts in numerous field and greenhouse experiments, and has been fully resistant to 29 races of loose smut and 14 of covered smut. It also is markedly resistant to almost all races of crown rust (*Puccinia coronata avenae*).

A specimen of loose smut (Collection 50) from Fulghum oats, collected by the junior writer at Stillwater, Oklahoma, in 1934, was sent to the Brooklyn Botanic Garden, where it was found to produce smut in Victoria and in certain hybrid strains derived from that variety.

Data on the infection of 22 selections from a Lee × Victoria cross are given in table 1.

The Lee × Victoria cross, from which these 22 promising selections were derived, was made by the junior writer in 1931. The selections listed were

TABLE 1.—*Reaction of Lee and Victoria oat varieties and selections from crosses between them to a newly discovered race of loose smut at the Brooklyn Botanic Garden, Brooklyn, N. Y., in 1941*

C. I. No.	Selection No.	Variety	Plants grown	Plants infected	
			Number	*Number*	*Per cent*
		Parental varieties			
2042	.....	Lee	27	25	92.6
2401	.....	Victoria	16	7	43.8
		Selections			
3379	P1–7–3–1	Lega	17	2	11.8
3384	P1–7–4–1	Levie	19	9	47.4
3392	P1–20–4–1	Letoria	24	12	50.0
3393	P5–9–3–2	Lenoir	19	11	57.9
3969	P32–4–1–2	.....	20	2	10.0
3400	P34–1–2–1	.....	15	1	6.6
3402	P34–1–2–3	.....	14	1	7.1
3404	P34–1–3–1	Lelina	21	10	47.6
3405	P34–1–3–2	.....	17	7	41.2
3406	P34–9–1–1	.....	25	17	68.0
3609	Coker 38–57	.....	23	11	47.8
3855	Coker 40–5	Stanton	10	2	20.0
3936	Coker 40–6	.....	28	6	21.4
3944	Coker 40–33	.....	23	9	39.1
3945	Coker 40–34	.....	18	10	55.6
3946	Coker 40–35	.....	24	9	37.5
3947	Coker 40–38	.....	22	10	45.5
3948	Coker 40–39	.....	29	13	44.8
3949	Coker 40–40	.....	13	7	53.8
3950	Coker 40–44	.....	16	6	37.5
3951	Coker 40–47	.....	22	4	18.2
3694	Ark. 3–28–12–1	.....	22	11	50.0

developed by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, in cooperation with the Iowa, North Carolina, Georgia, and Arkansas agricultural experiment stations and the Coker's Pedigreed Seed Company, Hartsville, South Carolina. These selections have been resistant to all other races of smut, and to most races of crown rust to which they have been subjected, and several of them showed such excellent performance that they were distributed to farmers.

Although preliminary results indicated that the Oklahoma smut collection was one of a half dozen known Fulghum-susceptible races of loose smut, it now seems evident that a hitherto unrecorded race of loose smut has been isolated. This race is especially interesting because of its pathogenicity for Victoria, thus far resistant to all known races of both oat smuts.

As shown in table 1, Victoria and Lee were infected 43.7 and 92.5 per cent, respectively. Lee is highly susceptible to many races of both smuts. The 22 selections showed infections by this new race of smut, ranging from 6.6 to 68.0 per cent. This new race may not be widely distributed, and it can only be hoped that many years may elapse before it seriously affects the importance of these new oats in the South.

Strains from other crosses with Victoria that are resistant to other races of smut also were tested for reaction to the new race. Three selections from the cross with Hairy Culberson showed 52.3 to 70.8 per cent of infection

when inoculated with the Oklahoma race. On the other hand, selections from crosses of Victoria with Fulgrain, Norton, and Nortex proved resistant. Nortex appears resistant, as also do Ranger and Rustler, which were selected from the Nortex-Victoria cross. Fultex was resistant to Collection 50 in the initial tests, although both Fulghum and Victoria, the parents of Fultex, were somewhat susceptible to the new race. It is probable that these two varieties carry different factors for resistance that combine to evolve resistant lines. Preliminary tests showed that the so-called standard smut-resistant varieties—Bond, Markton, and Navarro—were resistant to this new race. Black Mesdag, susceptible to certain other races, also was resistant. Red Rustproof appears to be resistant.

Further tests are necessary to determine the reaction to this new race of smut of other varieties and selections from Victoria crosses, such as Boone, Tama, and Vicland recently developed, important varieties.

The new race of smut, embraced by Collection 50 from Oklahoma, will be designated as A–30.—GEORGE M. REED, Brooklyn Botanic Garden, and T. R. STANTON, Bureau of Plant Industry, U. S. Department of Agriculture.